EVOLUTION OF MANKIND

IN his speech opening the debate on science in civil life in the House of Lords on December 9, Lord Taylor, in referring to the vanishing difference between pure and applied science, quoted both from Lord Adrian's recent Fawley Lecture on November 17 to the University of Southampton on "The Risks of Progress" and from Prof. P. B. Medawar's Reith Lecture on "The Future of Man". Lord Adrian remarked that we have become more and more aware that concentration on practical issues may lead to big theoretical advances; and he added that there is no longer any stigma attached to an idea or technique derived from a workshop rather than a university. Lord Taylor observed that applied science might lead to unexpected fundamental discovery, and pure science might yield utilitarian results when least intended; nevertheless, the difference remains, and in applied science someone must indicate the objective.

Considering more particularly medical science, Lord Taylor referred to the growing importance of producing health and fitness, but he used the term fitness in a rather wider sense than that of 'net reproductive advantage' used by Prof. Medawar in his second Reith Lecture, though Lord Taylor did not deny the importance of this definition in long-term genetic study. In this second lecture, Prof. Medawar considered the question whether man could go on evolving in the future as he has evolved in the past, or whether there is a good reason why his evolution should now have come to an end, and then asked, as a subsidiary question, whether there is any real reason for supposing that advances in medicine and hygiene are undermining the fitness of the human race. It is in this context that Prof. Medawar uses the word 'fitness' in the sense of 'net reproductive advantage', as it is generally used by students of heredity, though he recognizes that it neglects much that is deeply important in human life.

It may be recalled that in his first lecture, Prof. Medawar had emphasized the fallibility and limitations of our efforts to predict the future. Nevertheless, he maintained in his second lecture that it is not true to say that advances in medicine and hygiene must cause a genetical deterioration of mankind; there is more to be feared from a slow decline of human intelligence. But if that is happening, it is because the rather stupid are biologically fitter than those who are innately more intelligent, not because medicine is striving to raise the biological fitness of those people who might otherwise be hopelessly unfit.

This relation between intelligence and fertility was discussed more particularly in his fifth lecture, in which Prof. Medawar pointed out that while the average level of intelligence may in fact decline, this is no more than a presumption, first because one highly important piece of information, about the intelligence of married couples who have no children, or of people who never marry at all, is missing, and secondly, because we do not yet know exactly how differences of intelligence are inherited. Moreover, profound changes in fertility have been taking place during the past fifty or a hundred years, and they are not yet complete. Any decline of intelligence may be a purely temporary phenomenon, marking the transition from free reproduction accompanied by high mortality to restricted reproduction accompanied by low mortality.

There may thus be less difference between Prof. Medawar's views and that expressed by Lord Adrian in his Fawley Lecture than appears at first sight, when we contrast Prof. Medawar's words with Lord Adrian's balance sheet. Lord Adrian, too, emphasizes the need for much more information, and he himself suggests that we need not be too downcast at the picture he presented of the result of indiscriminate life-saving. We have succeeded so well, he observes, in our aim at keeping alive every child that is born that we are certainly preserving many unfavourable genes which would otherwise have died out. "If we set out to save the unfit we must expect more unfitness in the world and more inheritance of the factors which promote it. Even if the radiation level remains as it now is, the advance of science can harm the genetic constitution of the race".

Lord Adrian goes on to point out that even a doubling of the mutation-rate, however, would not double the load of inherited weakness until many generations had elapsed, for many of the faulty genes will be recessive or sex-linked. In the next thirty years we can confidently expect a great increase in our understanding of the ultimate structure of living matter, of viruses and chromosomes and of the whole molecular mechanism of inheritance and growth. While it would be idle to look further ahead than one generation, Lord Adrian is confident that at the present rate of progress the medical science of our great-grandchildren will make them far more capable of preserving the race. Moreover, Prof. K. Mather, in a recent broadcast on heredity and environment, has stressed the way in which human ideas and the power to record and communicate them make our social evolution a far more rapid process than our biological evolution. The effects of new knowledge take far less time to be established than those of a new mutation, and while the prospect of decline in the level of human ability is far more disturbing than that of a rise, Lord Adrian believes that our increased knowledge will enable us to find a way to escape the genetic dangers which may threaten us before our stock has been seriously harmed.

That, however, does not give a fair picture of Lord Adrian's approach to this question of the risks of progress. He began by directing attention to the way in which academic science and its relation to tech-

nology and industry have been violently forced out of the pattern which T. H. Huxley prescribed for it in the Victorian period. We have become painfully aware of the risks we may be running in allowing free play to all the developments of science in the technological field, and while most of us would be shocked at the suggestion of banning any attempt to pursue a line of scientific inquiry which aimed primarily at increasing our understanding of the natural world, most of us would cheerfully agree to the prohibition of work on certain practical developments if we thought they might have a harmful effect on the human race.

It is at this point that Lord Adrian brought this distinction between pure and applied science sharply into focus on the problems of the under-developed territories of the world. Much remains to be done before those territories can be freed from poverty and hard labour; but the objective cannot be attained if the timing is not adequately supervised, if the population of the world is allowed to increase more rapidly than we can increase our means for feeding and housing it, if we exhaust our stores of metals, coal and oil before we have solved the problem of fusing hydrogen atoms. Similarly, disaster may follow if we press ahead too rapidly along particular lines in particular parts of the world without reference to the general welfare, and our distinction between primary and secondary needs must take increasing account of the human requirements in increased leisure. The risks of progress, Lord Adrian urged, should be calculated risks, based on two principles: our concern about the risk of serious disease rather than the risk of injury, and our concern that the human race should remain at least as healthy and intelligent as it is now.

Lord Adrian illustrated his point in a general way by referring to the radiation hazard. Thus he remarked that there will be many countries using nuclear power which may be less careful than ourselves in the disposal of radioactive wastes, and in seeing that fires and explosions do not generate clouds of radioactive material. Precautions taken in Britain will not necessarily protect us from the results of large-scale disasters elsewhere, and it is especially in the context of the aid to the under-developed countries that we should begin, as Lord Adrian suggested, to consider what risks a civilized community should regard as reasonable to accept. Indeed, as should already be apparent, the present risks which this age confronts arise largely out of the disparities which exist in the societies which constitute civilization, whether within or across national frontiers, and the stresses which such disparities in standards of living or in the capacity for adaptation and assimilation of technical advance are liable to cause. With the increasing attention now being focused on Western aid to the under-developed countries, either technical or economic, it is the more important that due thought should be given to this aspect, and that technical assistance should be considered realistically and impartially in its widest context.

STELLAR POPULATIONS

Stellar Populations

Proceedings of the Conference sponsored by the Pontifical Academy of Sciences and the Vatican Observatory, May 20–28, 1957. Edited by D. J. K. O'Connell, S.J. Pp. lxv+544. (Castel Gandolfo: Specola Vaticana, 1958. Distributed by North Holland Publishing Company, Amsterdam; and Interscience Publishers, Inc., New York.) 76s.

THIS is not a text-book; neither is it merely a collection of independent papers on a common theme. It is the report of a successful conference held at the Vatican Observatory in 1957 on the complicated and rapidly changing problem of stellar populations.

The fundamental questions are how and when were the stars formed, how did the Milky Way come into being and what has determined its structure as we see it to-day? If we could sort out all the stars around us into groups or populations, according to age and origin, we should fairly certainly be much further on than now in our understanding of these larger questions. Only fifteen years have passed since Baade announced his now famous hypothesis that all stars belong to one of two families: Population I, the members of which are comparatively young, and are associated with interstellar matter and the spiral arms of galaxies; Population II, comparatively old and to be found in places where interstellar matter is scarce or non-existent, for example, the globular clusters, the elliptical galaxies, the nuclei of spiral nebulæ. Since then general opinion has come gradually to the view that the concept of two clear-cut populations is an over-simplification, and a good deal of time at this conference was taken up with producing a more acceptable scheme, not too elaborate in relation to the still very elementary nature of our knowledge.

The reader of "Stellar Populations", if he is to get much from the book, needs some idea of the underlying thoughts, not very explicitly described, which form the background of such discussions to-day. It is unlikely that all the participants had exactly the same picture in mind, and perhaps it is rash to attempt to put it into words. Although no fairy story, it could well start off with the familiar phrase 'once upon a time'; for the time-scale of the earlier stages of the Milky Way's history is still extremely uncertain.

Anyway, at this remote epoch there is supposed to have been a primitive diffuse gas of practically pure hydrogen. Somehow or other, by processes we do not properly understand, this gas condensed into first-generation stars (Baade's Population II), and at or about the same time the stars and gas condensed into a Milky Way system, not necessarily closely similar to our present Milky Way. This may have happened 5–10 thousand million years ago. The more massive of the first generation stars evolved rapidly, 'burned' their hydrogen into helium and later their helium into heavier elements. Theory shows us how this could happen, although only the fringe of the problem of computing the life-histories of appropriate star models has yet been tackled, but very significantly we have here a possible mechanism for the origin of the heavier elements. Some of the more rapidly evolving stars became unstable and either by catas-

trophic explosions or by shedding material from their outer layers (both processes can be observed to-day) 'enriched' the interstellar gas with helium and heavier elements as well as hydrogen. Afterwards there was more star-building from the interstellar gas. We are uncertain when this occurred, whether during critical periods when conditions were specially favourable, or at a more or less steady rate throughout the history of the Milky Way. We feel confident that some star formation has continued until astronomically very recent times, 1–10 million years ago (for Baade's Population I), and is probably still continuing to-day. During this history of perhaps 10^{10} years, the Milky Way evolved into the system we are now trying to explore by various techniques: a thin, flat, slowly rotating disk of stars and interstellar matter, with some spiral structure associated with dust, gas and young stars, and a more uniform background of older stars. The younger stars and the interstellar gas rotate in approximately circular orbits about the centre of the system; older stars tend to follow more elongated orbits, roughly elliptical, taking them at times near the centre. Around the centre older stars predominate and the system is supposed to have a nucleus and a central bulge similar to those we see in many external galaxies. Our picture includes also a very low-density halo, mainly gaseous but with a few stars, roughly spherical and for the most part detectable only by radio methods. The connexion of this halo with the rest of the system is so far not at all clearly understood.

Our ideas as to how all this came about are in detail still very vague. It is not unlikely that some essential parts of the problem have still eluded us, but so many different lines of evidence tend to point in the same general direction that we are persuaded that roughly we are thinking along the right lines. The problem of stellar populations is to sort out the stars as we see them to-day into some kind of an age order, and then to study their other physical properties in relation to this. We try to do this by using positions in the H–R. diagram, velocities, colour characteristics, or spectra, depending on what is available. The measurements are generally tedious, they often need large telescopes, and even when obtained the results may be inconclusive or ambiguous. Theoreticians have tasks as formidable as those of observers. For example, one of the conference members (Hoyle) estimated that something of the order of 10,000 hr. would be necessary on the largest existing electronic computers, to calculate the life-histories of a reasonably representative set of star models. Clearly, we are unlikely to get quick answers to any of the bigger questions, and since this kind of astronomical problem usually grows in complexity as our knowledge increases, the final answers may yet be a very long way off.

Meanwhile, here is a picture of the subject as it was in 1957, not a neat text-book account for a beginner, but a report of research in progress, with untidy ends everywhere, full of food for thought, but also capable of stirring up dissatisfaction, even irritation, in a critical reader. The organizers of this conference had the wisdom to keep it small. Among other great advantages this kept the discussions informal, spontaneous, well-informed and relevant. The reporting of both papers and discussions appears to have been done excellently.

R. O. REDMAN

HISTORY OF SCIENCE

A Short History of Scientific Ideas to 1900

By Dr. Charles Singer. Pp. xviii+525. (Oxford: Clarendon Press; London: Oxford University Press, 1959.) 35*s*. net.

WHEN Dr. Singer's "Short History of Science to the Nineteenth Century" appeared in 1941 it was the first, in the English language and within a reasonable compass, to "present, in simple form, the development of the conception of a rational and interconnected material world"; to relate the particular activity we call 'science' to the remaining intellectual activities of the heirs of Greco-Roman civilization. To emphasize the progressively changing character of that relationship the author introduced such leading phrases as "The Great Adventure" (Athens), "The Failure of Nerve" (Alexandria), "The Failure of Inspiration" (Rome), "The Failure of Knowledge" (Middle Ages), "The Revival of Learning", "The Insurgent Century", and "The Mechanical World". The present version—Dr. Singer rightly claims that it is "much more than a revision and rather more than a new edition"—contains in addition a succinct account (contributed by Dr. Angus Armitage) of the earliest records of systematized knowledge in the Middle East, and an additional Section—"Culmination of the Mechanical View of the World"—running to nearly 100 pages, rather less than half of which have been written by Prof. H. Dingle.

The book has been further enriched by two contributions by Dr. Derek Price, one on the work and instruments of Ptolemy, the other on the plane astrolabe. A few sub-sections, notably one on the "Status of Medieval Science", have been added, and others (for example, on the sixteenth century herbalists and anatomists) largely re-written. The importance of technological change is everywhere brought into greater prominence, and this is reflected among the much larger number of admirable illustrations, for the use of many of which, from "A History of Technology", Dr. Singer expresses gratitude to the Directors of Imperial Chemical Industries, Ltd.

The original "Short History", admirable as it was, could perhaps be criticized on the grounds of its sweeping condemnation of medieval science as a "period of intellectual degradation" (p. 161); the lack of balance in respect of the history of chemistry; and but a scant reference to embryology. Whereas the last lack has now been made good, it seems regrettable that, with the change of title and with the wealth of studies on the medieval period since the first appearance of the earlier version, the opportunity was not taken to present the medieval architects of ideas in a more sympathetic light. In regard to chemistry the situation has actually deteriorated. Though Prof. Dingle graciously allows that "the history of physics for a long time to come will not be without a chemical chapter" his ingeniously phrased "oraison funèbre", though not without expressions of profound respect for the departed, will scarcely pass as 'history'. Frankland did not just "assign" to each atom a property called valency; he was driven to speak (rather apologetically) of a "saturation capacity" in consequence of results unexpectedly obtained in his efforts to isolate a compound radical. To ignore the paths by which the Pisgah-views, now shared and interpreted by theoret-

ical physicists, have been reached is to nullify a great deal of the cultural value of the history of science.

The faults, however, cannot outweigh the delight which awaits the reader in the many wise comments and illuminating comparisons scattered throughout the whole book. Perhaps, when in a few years time a third version is called for, the master from whom we have all learned so much might consider the dropping of the catch-titles. These may have been justified when first introduced; but has not the time now come when a history of science should place the emphasis on *wie es eigentlich gewesen*?

William P. D. Wightman

AGRICULTURAL RESEARCH IN INDIA

Agriculture and Animal Husbandry in India

By Dr. M. S. Randhawa. Pp. xvi+364. (New Delhi: Indian Council of Agricultural Research, 1958.) Rs. 15.00.

Mechanical Cultivation in India

By D. A. Gadkary. Pp. ix+147+10 plates. (New Delhi: Indian Council of Agricultural Research, 1957.) Rs. 7.25; 11*s*. 6*d*.

AGRICULTURAL research in India is a subject of such importance that it is scarcely an exaggeration to say that the future of the world may depend upon it. Indian scientists and the Indian Council of Agricultural Research have had quite a good record so far, though both the financial and human resources of India fall far short of the real requirements of the situation. As Dr. Randhawa freely admits, other countries faced with the problem of obtaining maximum food supply from a limited area—Japan, Italy and Egypt—obtain yields per acre three or four times India's. If India could raise her yields to these levels she could over-feed any conceivable population she is likely to have for a very long time to come. But mere transplantation of seeds, fertilizers and methods of cultivation from, say, Japan, will not suffice. Plant breeding, as optimistic farmers have found to their cost, is a laborious task which has to be done over again for even the slightest difference in climate, and then again as new pests appear.

"Agriculture and Animal Husbandry in India" does not deal with the problems on the actual frontier of research, though the compilers are clearly aware of them. It is a compendium of information on the best strains of plants now available, cultivation requirements, expected yields, and where the seeds can be obtained. The geographical maps and statistical tables have been well done, though the chapter on irrigation is disappointingly superficial, and livestock receive much less attention than crops.

For every scientist engaged in original agricultural research India will need perhaps a hundred men to disseminate his results, as well as the elements of agricultural science, among an uninformed people. A book like this should play a valuable part in their training, and be a useful reference book to librarians in other countries.

"Mechanical Cultivation in India" is not broad enough in its scope to constitute an economic study of the circumstances (admittedly limited) in which mechanical cultivation is now worth introducing in India. Nor is it detailed enough to serve as an operator's handbook. It appears to have been designed more as an administrative handbook, imparting the necessary minimum of information about types of machines, and making preparations for the administrative, recording and accounting duties which will arise if mechanical implements are to be operated from governmental tractor stations.

Colin Clark

INDUSTRIAL GUMS

Industrial Gums

Polysaccharides and Their Derivatives. Edited by Roy L. Whistler and James N. BeMiller. Pp. xi+766. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 25 dollars.

BRIDGING the gap between the academic chemist and the chemical technologist is always a problem since in general their points of view differ widely. Here it has been achieved by competent academic editing of a series of chapters written mainly by industrial chemists. The brief 'academic' chemistry of gums included in the book does not interfere, and is indeed necessary, for the main task of presenting detailed information of those practical applications of gums which are of particular interest to food technologists, paint and textile manufacturers, paper industrialists and pharmaceutical chemists. The current prices and potential resources of many of the gums are included. Certain gums which, as yet, have not been used widely in industry are included, together with suggestions as to their possible applications.

My minor criticisms of this book include a lack of consistency from one chapter to another in depiction of polysaccharide structures and indeed their complete absence in certain chapters. In the otherwise competent chapter on dextran I could find no details of the Swedish manufacture of dextran or of the use of iron complexes of dextran. The author was also unaware that the structure of the mycodextran from *Aspergillus niger* (p. 552) had been extensively investigated and that it had been renamed nigeran, due to its marked structural difference from normal dextrans. The more detailed formula for laminaran postulated by Peat and his colleagues could well have been included. In the chapter on agar no mention is made of its application in the now widely used agar diffusion technique. The authors of the agar article are employed by the only American company manufacturing agar, and can perhaps be forgiven for advertising the superiority of their company's product as opposed to that of the much cheaper Japanese agar. The book gives detailed coverage of the literature up to the end of 1957 with scattered references from 1958.

I can thoroughly recommend this book as essential for all technical libraries, providing as it does comprehensive references to the numerous patents on the industrial uses of gums. I am sure it will be read more widely than its title suggests, since by analogy numerous applications can be suggested for many unexploited polymers, particularly as attempts are made throughout the book to relate the physical characteristics of the gums to their chemical structure. After editing this book, Prof. Whistler will be much sought after as an industrial consultant.

S. A. Barker

Radioisotopes in Scientific Research

Proceedings of the International Conference held in Paris in September 1957, under the auspices of the United Nations Educational, Scientific and Cultural Organization. Edited by Prof. R. C. Extermann. Vol. 1: Research with Radioisotopes in Physics and Industry. Pp. xxi+761. Vol. 2: Research with Radioisotopes in Chemistry and Geology. Pp. xxi+741. (London and New York: Pergamon Press, 1958.) 140*s*. net each volume.

THE Unesco Conference on "Radioisotopes in Scientific Research" has been reported in *Nature*, 180, 1029 (1957). The proceedings, which have now been published, comprise the contributions in their original language, but the abstracts of each paper are given in English, French, Russian and Spanish. The discussions are reported for each paper, and, although greatly reduced in volume, are well edited. Contrary to general belief that such research outdates itself in a reasonably short time, one has to admit that some of the material presented will be extremely useful to workers in this field for many years to come. For example, the work reported on the tracing of water by using cosmic-ray and man-made tritium, by Begemann and Libby, is only now fully appreciated, and work in years to come will be based on the ideas contained in this paper. The proceedings of this most useful conference should certainly be in every science library and, to a certain extent, they can be regarded as reference books.

Although the reproduction and printing are reasonable, if not in any way elaborate, the price of the volumes seems rather high. It is a pity that the book, which came out one year after the conference was held, still contains a number of misprints, the most misleading ones being found in the index of Vol. 2, where twenty-two contributions have been indexed with the wrong page number owing to the fact that one contribution was erroneously split in two, whereas at the end of the book the page number again agrees with that of the papers, because one page number was left out for one contribution altogether.

H. SELIGMAN

Nitrogen Metabolism in Plants

By Prof. George C. Webster. (Row–Peterson Biological Monographs.) Pp. viii+152. (Evanston, Ill.: Row, Peterson and Company, 1959.) 3.50 dollars.

THIS is an age in which authoritative reviews in the fields of plant physiology and plant biochemistry are tending to be concentrated in encyclopædias or other massive volumes which are too expensive for individual ownership; indeed, the influence of such compilations on scientific thought may prove to be limited because of the physical difficulty of consulting them. Thus, there is still very much room for smaller publications in which substantial fields of investigation are reviewed authoritatively and, though not exhaustively, with sufficient documentation for the reader to be made aware of the more important original sources. Such a review is provided by this very useful little book, which is concerned mainly with the chemical pathways of nitrogen metabolism in plants and has been written by an author who is already well known for his own original studies in this field. The topics covered are nitrogen nutrition, amino-acid metabolism, biosynthesis of amides and peptides, protein and nucleic acid metabolism, nitrogen metabolism of excised organs and ageing tissues, and translocation of nitrogenous substances. The book is written in straightforward and clear style, marred only occasionally by infelicitous phrases or repetitive treatment. In a few instances (for example, Table 2) the identity of the plant material to which the data presented refer is not stated; three misprints were noted, while an arrow appears to be missing from Fig. 26. These are minor blemishes in an otherwise workmanlike text, which I recommend for use by research workers and honours students.

G. BOND

Bird Watching for Beginners

By Bruce Campbell. (Puffin Book PS71.) Pp. 240. (Harmondsworth, Mddx.: Penguin Books, Ltd., 1959.) 2*s*. 6*d*.

THIS is a revised edition of a book first published in 1952. It is divided into three sections. The first explains how to become a bird watcher and the limits of classification. The second discusses two hundred common British resident and visiting species in systematic order from crows to game-birds. It deals mainly with relationships, distribution and habits, and points out aspects "suitable for study by the ordinary bird watcher".

Part 3 briefly surveys various problems; how to set about identification, how to make bird counts, how to study birds at the nest, and so on.

The author is highly qualified to give instructions to beginning bird watchers. He has an extensive practical knowledge of field-study and his association with the British Trust for Ornithology has given him a wide background of the subject based on the experience of others. Ability to impart this knowledge has been gained from long practice as lecturer and broadcaster. He has a readable style and pleasing personal approach which not only provides information but also inspires enthusiasm.

J. D. MACDONALD

The Rhondda Valleys

A Study in Industrial Development, 1800 to the Present Day. By E. D. Lewis. Pp. xiii+312+3 plates. (London: Phoenix House, Ltd., 1959.) 25*s*. net.

THE Rhondda became a borough in 1955 and to mark the event sponsored the publication of this excellent local history. Begun twenty years before as a master's thesis for the University of Wales, its author, the son of a Rhondda collier and now a Rhondda headmaster, has expanded his work to a scholarly and well-documented local history. The story of the Rhondda is well known, being that of a sparsely populated, picturesque, well-wooded valley, rarely penetrated by strangers at the beginning of the nineteenth century; it passed through a whole cycle of industrial development within the next hundred years, to be hit with more than average severity by the depression following the First World War. What would perhaps, in another continent and another context, have become a ghost town as its *raison d'être* faded, had too great a reservoir of human vitality to succumb. Much of the book is devoted to the human story—the early working conditions, the rise and temporary fall of trade unionism, and the later role of the Rhondda as stormy pioneer and leader in new concepts of management and labour relationships. The final chapter, dealing with the period from 1914 to the present day, makes it clear that the story is far from ended.

L. DUDLEY STAMP

STRUCTURE OF HÆMOGLOBIN

A THREE-DIMENSIONAL FOURIER SYNTHESIS AT 5·5-Å. RESOLUTION, OBTAINED BY X-RAY ANALYSIS

By Dr. M. F. PERUTZ, F.R.S., Dr. M. G. ROSSMANN, ANN F. CULLIS, HILARY MUIRHEAD and Dr. GEORG WILL

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

AND

Dr. A. C. T. NORTH

Medical Research Council External Staff, Davy Faraday Research Laboratory, Royal Institution, London, W.1

VERTEBRATE hæmoglobin is a protein of molecular weight 67,000. Four of its 10,000 atoms are iron atoms which are combined with protoporphyrin to form four hæm groups. The remaining atoms are in four polypeptide chains of roughly equal size, which are identical in pairs[1–3]. Their amino-acid sequence is still largely unknown.

We have used horse oxy- or met-hæmoglobin because it crystallizes in a form especially suited for X-ray analysis, and employed the method of isomorphous replacement with heavy atoms to determine the phase angles of the diffracted rays[4–7]. The Fourier synthesis which we have calculated shows that hæmoglobin consists of four sub-units in a tetrahedral array and that each sub-unit closely resembles Kendrew's model of sperm whale myoglobin[8]. The four hæm groups lie in separate pockets on the surface of the molecule.

Method of Analysis

Horse oxyhæmoglobin, crystallized from 1·9 *M* ammonium sulphate solution at *p*H 7, has the space group *C*2 with two molecules in the unit cell which lie on dyad axes[9]. In order to determine the phase angles of the 1,200 reflexions contained in the limiting sphere of 5·5 Å.$^{-1}$, six different isomorphous heavy-atom compounds were used (ref. 9 and unpublished work). Intensities were measured photographically and by counter spectrometer (Arndt, U. W., and Phillips, D. C., unpublished work). The relative positions and shapes of the heavy-atom replacement groups were found by correlation functions based on Patterson methods[10] and refined by least squares procedures. For each reflexion the structure amplitudes of all seven compounds were combined in an Argand diagram[11], and the probability of the phase angle having a value α was calculated for $\alpha = 0, 5, 10, \ldots 355°$. The centroid of the probability distribution, plotted around a circle, was then chosen as the best vector F in the Fourier synthesis[12]. The results were finally plotted on 32 contour maps showing the distribution of electron density in sections spaced 2 Å. apart normal to *b* (Fig. 1). The absolute configuration of the molecule was determined from anomalous dispersion[4].

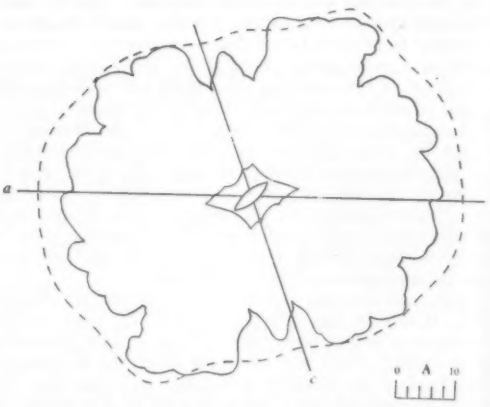


Fig. 2. External shape of the molecule. Full lines indicate the boundary derived from the contour at 0·54 electron/Å.^{3}. The broken line shows the boundary derived by Bragg and Perutz from two-dimensional data (ref. 13). Note the hole in the middle

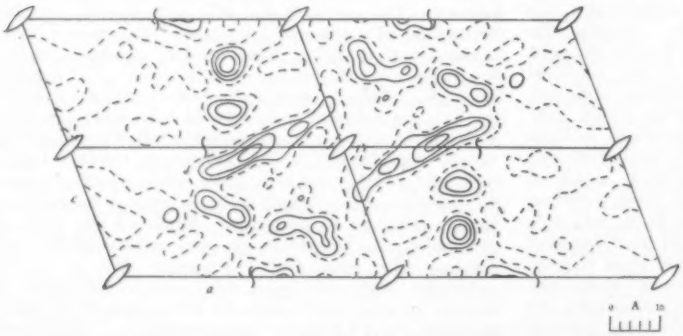


Fig. 1. Section at $y = 1/32b$. This cuts through the middle of the molecule on which the diagram is centred. 'Flat' areas indicating liquid appear on the left and right. Contours are drawn at intervals of 0·14 electron/Å.^{3}. The broken line marks 0·4 electron/Å.^{3}. Contours at lower levels are omitted

External Shape of the Molecule

More than half the volume of the crystals is taken up by liquid of crystallization, which mainly fills the spaces between the molecules and shows up on the contour maps in the form of flat, featureless regions (Fig. 1). The outlines of the two molecules in the unit cell can be traced by following the boundaries between these regions and the continuous electron-dense regions described below. In Fig. 2 the outline of one molecule, traced from the periphery of the 0·54

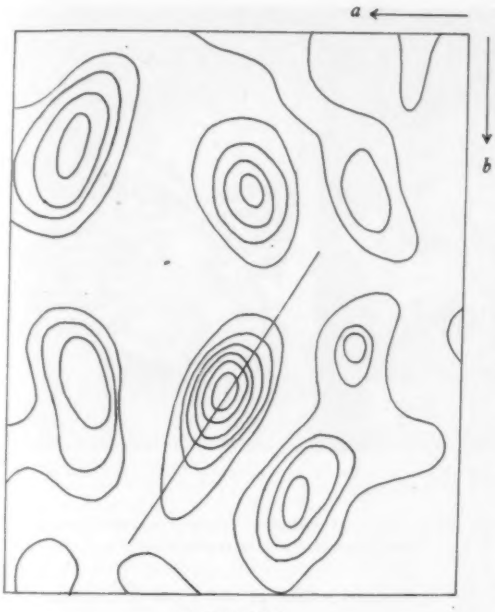


Fig. 3. Section at $z = 1/4$ *c* showing one of the two hæm groups in the asymmetric unit. The straight line indicates the hæm orientation derived from electron spin resonance (ref. 14). The lowest contour shown is at 0·4 electron/Å.^{3}

electron/Å.^{3} contour, is seen in projection on the *b*-plane. To a first approximation it can be regarded as a spheroid with a length of 64 Å., a width of 55 Å. and a height of 50 Å. normal to the plane of the paper. Except for a slight shortening along *a*, this shape agrees with the earlier picture obtained from two-dimensional data, even including the dimple in the centre of the molecule (see Fig. 2 of ref. 12).

Positions of the Hæm and Sulphydryl Groups

Four peaks stand out from the rest, clearly representing the iron atoms with their surrounding porphyrin rings. A flattening of the peaks indicates the approximate orientation of the rings (Fig. 3). Fortunately, their exact orientation was already known from electron spin resonance[14], and it only remained to assign the correct one of the four alternative orientations of the hæms to each of the electron density peaks. The results are shown in Fig. 4. The iron atoms lie at the corners of an irregular tetrahedron with distances of 33·4 and 36·0 Å. between symmetrically related pairs. The closest approach between symmetrically unrelated iron atoms is 25·2 Å. (Table 1).

Table 1

	Fe_1	Fe_2	S_1
x	−6·6 Å.	12·3 Å.	5 Å.
y	7·3 Å.	−10·7 Å.	10 Å.
z	13·1 Å.	18·2 Å.	16 Å.

$Fe_1 - Fe_2 = 33{\cdot}4$ Å.
$Fe_2 - Fe_4 = 36{\cdot}0$ Å.
$Fe_1 - Fe_3 = 25{\cdot}2$ Å.
$Fe_1 - S_1$ 13 Å.
$Fe_2 - S_1$ 21 Å.

Horse hæmoglobin contains four cysteine residues, but only two sulphydryls combine with mercury in the native protein[5]. From the positions of the mercury atoms we inferred that each of the two sulphydryl groups is about 13 Å. away from one iron atom and 21 Å. away from another. (Fig. 4 and Table 1). The significance of this situation is discussed below.

Configuration of the Polypeptide Chains

The most prominent feature of the Fourier synthesis consists of more or less cylindrical clouds of high density, like the vapour trails of an aeroplane; they are curved to form intricate three-dimensional figures. Sections through various parts of these appear in Fig. 1. To build a model of the figures, we rolled out sheets of a thermo-setting plastic to the thickness of our sections on a scale of 2 Å. = 1 cm., and cut out the shape of each region on the contour maps where the density exceeds 0·54 electron/Å.^{3} (this corresponds to the first full contour line in Fig. 1. From this section, for example, 14 shapes would be cut). The shapes were then assembled in accordance with their positions and heights in the different sections, and the hæm groups were attached in the appropriate orientation. The model was then baked to set it permanently. For comparison, a Fourier synthesis of sperm whale myoglobin was calculated at a resolution of 5·5 Å., using the new X-ray data of Kendrew *et al.*[15], and a model of the electron density distribution was constructed by the method just described.

From the hæmoglobin Fourier synthesis there emerged four separate units which are identical in pairs. Fig. 5 shows one member of each pair together with the model of myoglobin on the left. In each unit the cloud of high density describes a complicated figure which, in the white unit (middle), can be traced from end to end by following the superimposed line. Except for two small gaps where the density sinks slightly below 0·54 electron/Å.^{3}, the black unit (right) closely resembles the white one. There are several gaps interrupting the myoglobin model where, probably due to increased thermal motion, the electron density falls below the contour-level chosen for cutting the model. However, we know from the recent work of Kendrew and his collaborators[15] that

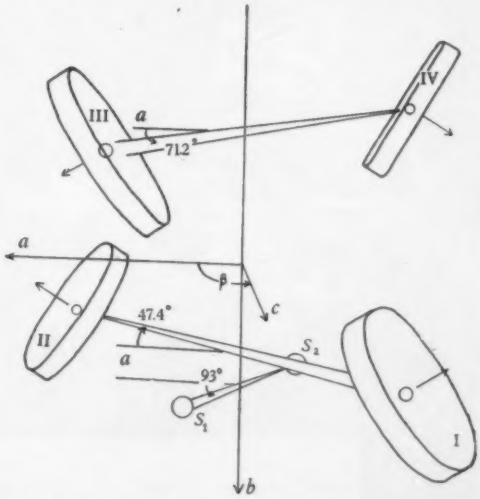


Fig. 4. Arrangement of hæm groups in hæmoglobin. Arrows indicate the reactive side of each group. The *c*-axis comes out of the paper towards the observer. This picture and all subsequent ones show the absolute configuration

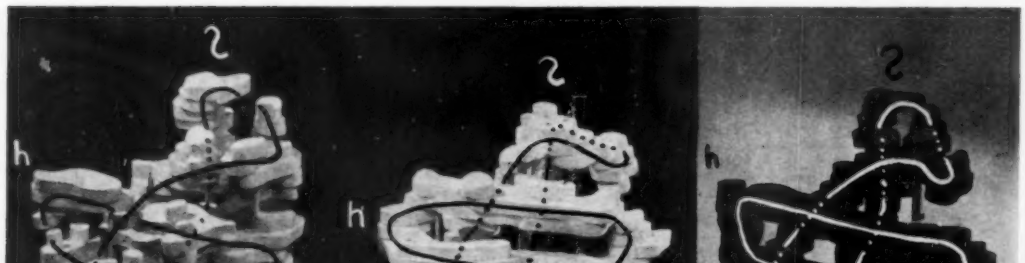


Fig. 5. Two different polypeptide chains in the asymmetric unit of hæmoglobin compared with myoglobin (left). The hæm groups are at the back of the chains

the gaps are bridged by a continuous polypeptide chain, and it is evident from Fig. 5 that apart from the gaps, the model has a configuration closely similar to the hæmoglobin units.

Clearly, the four tortuous clouds of high electron density in hæmoglobin represent the four polypeptide chains. The black and the white chains have similar, but not identical configurations. In the black chain the *S*-shaped bend at the top is more pronounced, the hæm group is lower, and the bend *h* sharper than in the white chain. These, however, are details. The most important result is their resemblance to each other and to sperm whale myoglobin.

Arrangement of the Four Sub-units

The first step in the assembly of the molecule is the matching of each chain by its symmetrically related partner (Fig. 6). It will be noted that there is comparatively little contact between the members of each pair, suggesting rather tenuous linkages. In the next step the white pair is inverted and

Fig. 6. Two pairs of chains symmetrically related by the dyad axis. The arrow shows how one pair is placed over the other to assemble the complete molecule

placed over the black pair as indicated by the arrow. Fig. 7 shows one white chain placed over the pair of black ones and Fig. 8 shows the molecule completely assembled. The resulting arrangement is tetrahedral and has almost, but not quite, the orthorhombic point group symmetry 222. It contains two 'pseudo dyads' which lie approximately at right angles to each other and to the true dyad, one emerging from the centre of Fig. 8 and the other from the centre of Fig. 9. This means that, to a first approximation, each sub-unit can be generated by a rotation of 180° from any of its neighbours. Figs. 7, 8 and 9 also show that the surface contours of the white chains exactly fit those of the black, so that there is a large area of contact between them. This structural complementarity is one of the most striking features of the molecule. Fig. 10 is a view down the true dyad axis and reveals a hole going right through the centre of the molecule, as was to be expected from the Fourier projection[13]. However, the van der Waals radii of the chains are much bigger than appears in the model, and little room may, in fact, be left for water or electrolytes to pass through. Fig. 10 also reveals a dimple at the top where the white chains meet. This is matched by a similar, but larger, hollow at the bottom where the black chains meet.

Fig. 7. Partially assembled molecule showing two black chains and one white

The hæm groups are seen to lie in separate pockets on the surface of the molecule (Fig. 8). Each pocket is formed by the folds in one of the polypeptide chains, which appears to make contact with the hæm group at four different points at least. The iron atoms in the neighbouring pockets formed by the black and the white chains are 25 Å. apart.

Information from the Fourier Synthesis of Myoglobin at 2-Å. Resolution (ref. 15)

Thanks to the similarity with myoglobin, the interpretation can be carried further than would have been possible on the basis of our results alone. Kendrew *et al.* have found that the straight stretches of rod indicated in Fig. 5 are α-helices and that the N-terminal end of the chain is at the bottom left. The ends of the two hæmoglobin chains have been labelled *N* and *C* accordingly, as seen in Figs. 7 and 10. The myoglobin Fourier synthesis also reveals the hæm-linked amino-acid side-chain, probably histidine, on one side of the (ferric) iron atom and a small peak, probably representing a water molecule, on the other side. If this information is transferred to hæmoglobin, the reactive side of the hæm group is as indicated by the arrows in Fig. 4 and by the labels O_2 in Figs. 7 and 8*a*.

Figs. 7 and 8*b* show the reactive sulphydryl group (which is absent in myoglobin) to be attached to the portion of the black chain carrying the hæm-linked histidine. It is seen that the histidine and cysteine side-chains point in roughly opposite directions, one towards and the other away from the hæm group. The sulphydryl group may possibly be in contact with the loop of the white chain which lies below the hæm group on the left. This is the situation of one pair of sulphydryl groups in the molecule. The second pair is probably attached to the white chains, but it is unreactive in the native protein and its position is still unknown.

Reliability of Results

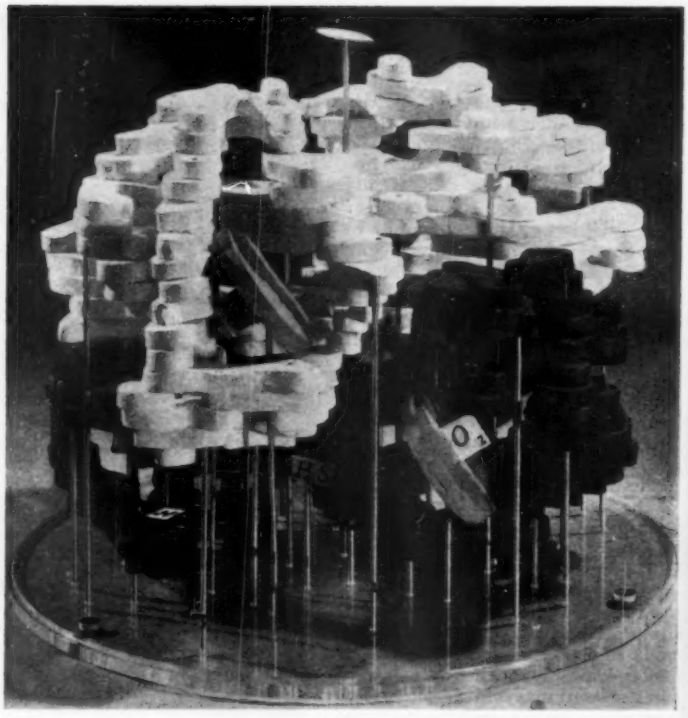
The method of isomorphous replacement makes no assumptions about the structure of the protein, and its results suffer from none of the ambiguities which bedevil the interpretation of vector maps. Ideally, the parent protein, in combination with two different isomorphous heavy-atom compounds, should give accurate phase angles and electron density maps which are free from all except series termination errors. In practice, errors and uncertainties arise from several sources and must be minimized by using more than two heavy-atom compounds. In our case it was possible to estimate the accuracy of the phase angle for each reflexion from the measure of agreement between the angles indicated by the six different compounds. From the standard error in the vector *F*, averaged over all reflexions, the standard error in the final electron density was calculated as 0·12 electron/Å.^{3}, which amounts to 0·85 of the interval between successive contours in Figs. 1 and 3 (0·14 electron/Å.^{3}), or 0·15 of the difference in density between 'peaks' and 'valleys' (0·7 electron/Å.^{3}) (ref. 12). This calculated error is borne out by the observed fluctuations in the liquid regions between the molecules and by the difference in height between the two iron peaks (0·13 electron/Å.^{3}).

An error of at least five times this magnitude would be needed to turn a peak into a valley and thus to simulate the backbone of a polypeptide chain in a region actually occupied by side-chains.

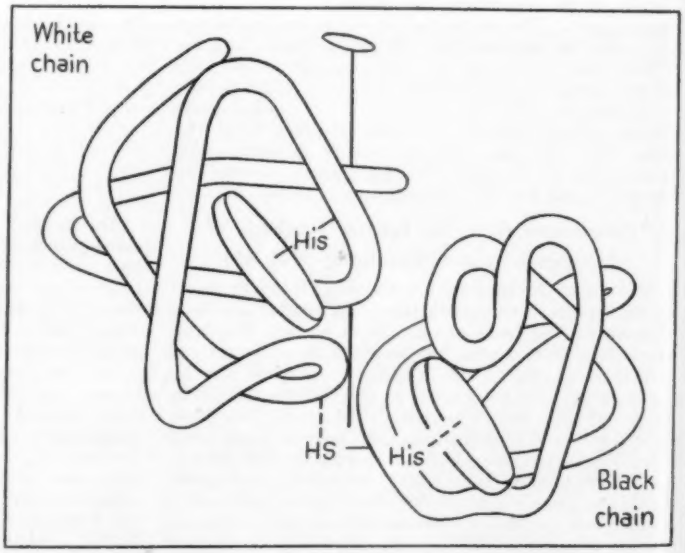
Normally, the accuracy of a structure is checked by comparison of calculated and observed intensities. In hæmoglobin this is impossible so long as the light atoms are not resolved, but the positions of the iron atoms at least can be checked by their anomalous dispersion effect. Depending on the phase relationship between the structure factor of the four iron atoms by themselves and that of the entire molecule, the total intensity of any given reflexion $I\ (hkl)$ may be larger or smaller than $I\ (\bar{h}\bar{k}\bar{l})$. We selected 50 reflexions for which the effect of anomalous dispersion was expected to be largest and measured the intensity of each reflexion in the four symmetrically related quadrants on the counter spectrometer. Statistically significant differences between $I(hkl)+I(\bar{h}\bar{k}\bar{l})$ and $I(\bar{h}\bar{k}l)+I(h\bar{k}\bar{l})$ were found in 36 reflexions. In 34 of them the signs of the differences agreed with prediction, indicating that the phase angles of the reflexions as well as the positions of the iron atoms are correct.

As a further check on the positions of the iron atoms, an attempt was made to label them with heavy atoms. This was done by allowing crystals of methæmoglobin, which are isomorphous with oxyhæmoglobin, to react with *p*-iodophenyl–hydroxylamine, which attaches itself to the iron atoms. A difference Fourier projection on the *b*-plane showed four prominent peaks which lie well within the calculated distances between the iodine and the iron atoms.

Finally, to avoid any possible bias in the interpretation of the Fourier synthesis, we have tried to construct objective models containing only those features actually found in the electron density maps. Due to series termination errors and inaccuracies in many of the phase angles, these maps must certainly contain errors of detail. Large errors, on the other hand, are unlikely because, quite apart from the checks just described, the results really prove themselves. No combination of errors could have led to the appearance of four distinct chains of roughly equal length, in agreement with chemical evidence, to the similarity between the two pairs of chains which are not related by crystal symmetry, and to the resemblance between hæmoglobin and myoglobin.



(*a*)



(*b*)

Fig. 8. (*a*) Hæmoglobin model viewed normal to *a*. The hæm groups are indicated by grey disks. (*b*) Chain configuration in the two sub-units facing the observer. The other two chains are produced by the operation of the dyad axis

Discussion

The polypeptide chain-fold which Kendrew and his collaborators first discovered in sperm whale myoglobin has since been found also in seal myoglobin[16].

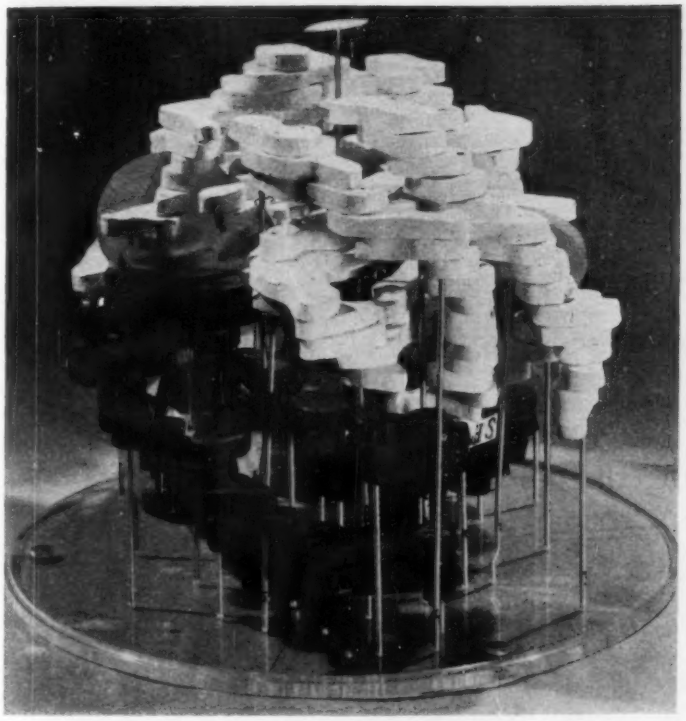


Fig. 9. Hæmoglobin model viewed normal to *c*

Its appearance in horse hæmoglobin suggests that all hæmoglobins and myoglobins of vertebrates follow the same pattern. How does this arise ? It is scarcely conceivable that a three-dimensional template forces the chain to take up this fold. More probably the chain, once it is synthesized and provided with a hæm group around which it can coil, takes up this configuration spontaneously, as the only one which satisfies the stereochemical requirements of its amino-acid sequence. This suggests the occurrence of similar sequences throughout this group of proteins, despite their marked differences in amino-acid content. This seems all the more likely, since their structural similarity suggests that they have developed from a common genetic precursor.

Little can be said as yet about the relation between structure and function. The hæm groups are much too far apart for the combination with oxygen of any one of them to affect the oxygen affinity of its neighbours directly. Whatever interaction between the hæm groups exists must be of a subtle and indirect kind that we cannot yet guess. A few observations of possible significance might be mentioned. Kendrew found that the combination of reduced myoglobin with oxygen involves no structural changes detectable by X-ray analysis[17], both forms being isomorphous with the metmyoglobin normally studied. In the hæmoglobin of horse and of man, on the other hand, the oxygenated and reduced forms are crystallographically different[18–20]. The structure of reduced hæmoglobin is still unknown, but it would not be surprising if loss of oxygen caused the four sub-units to rearrange themselves relative to each other, rather than to change their individual structure to a marked degree.

Riggs has shown that blocking the sulphydryl groups reduces hæm–hæm interaction[21]. As Fig. 8 shows, these groups occupy key positions close to the hæm-linked histidines and to points of contact between two different sub-units. They may well play an important part in the transition between the oxygenated and reduced forms. Incidentally, the cysteine residue should provide a convenient marker for the peptide containing the hæm-linked histidine, and so help to determine the sequence in this important part of the chain.

A full account of this work will be published elsewhere.

We thank the Director and staff of the University of Cambridge Mathematics Laboratory for making their electronic computor *Edsac* II available to us for the many calculations involved in this work. We are

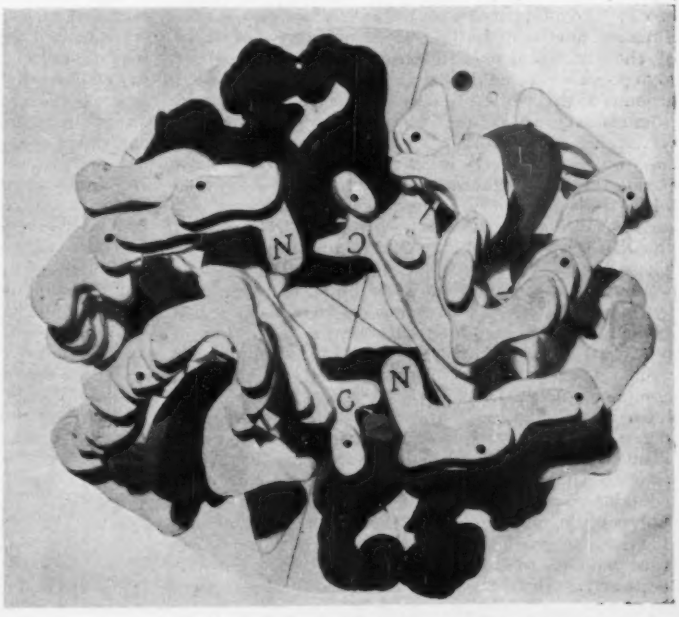


Fig. 10. A view down the *b*-axis. Note the proximity of the *C* and *N* terminal ends which could serve to form links between the two white chains

also deeply grateful to the Rockefeller Foundation for its long-continued financial support and to Sir Lawrence Bragg for his unfailing enthusiasm and encouragement. Finally, we wish to thank Dr. B. R. Baker, of the Stanford Research Institute, for a gift of organic mercurials, Dr. J. Chatt of Imperial Chemical Industries, Ltd., and Mr. A. R. Powell, of Johnson Matthey, Ltd., for gifts of heavy-atom compounds, and Mrs. Margaret Allen, Miss Ann Jury and Miss Brenda Davies for assistance.

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STRUCTURE OF MYOGLOBIN

A THREE-DIMENSIONAL FOURIER SYNTHESIS AT 2 Å. RESOLUTION

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MYOGLOBIN is a conjugated protein consisting of a single polypeptide chain of about 153 amino-acid residues associated with an iron–porphyrin complex, the hæm group; its molecular weight is about 18,000, and the molecule contains some 1,200 atoms (excluding hydrogen). Two years ago a preliminary report of the first stage of an investigation of the three-dimensional structure of sperm-whale myoglobin was published in *Nature*[1] (a detailed account of this work has appeared recently[2]). Several isomorphous crystalline derivatives of myoglobin containing heavy atoms (mercury or gold) at single sites on the molecule were prepared, and by comparing the X-ray diffraction patterns of these crystals with those of the unsubstituted protein, it was possible to deduce the phases of all the reflexions in the X-ray pattern having spacings greater than 6 Å. These phases, together with the observed amplitudes, were used to compute a three-dimensional Fourier synthesis of the electron density in the unit cell (which contains two molecules) at a resolution of 6 Å. In this synthesis the polypeptide chain was visible as a rod of high electron density, folded in a complex pattern (Fig. 5*a*); in addition, the single hæm group with its iron atom, which is much more dense than any other atom in the molecule, could be identified as a disk of high electron density. The shape of the molecule could be determined with some confidence, as could most of the course of the single polypeptide chain, though there were several ambiguities where it turned through a large angle, so that the ends of the chain could not be located with certainty. Thus, the general nature of the tertiary structure of the molecule was revealed, but not the secondary structure of the polypeptide chain, though the results were consistent with a helical configuration.

More recently, Scouloudi[3] used similar methods to obtain a two-dimensional Fourier projection of the unit cell of seal myoglobin, in which the molecular arrangement is entirely different from that in sperm-whale crystals; she was able to show that the myoglobins of these two species have essentially the same tertiary structure in spite of their different amino-acid compositions. Her work, by implication, confirmed the correctness of both analyses, as well as the deductions made about the shape of the molecule. In the accompanying article[4] Perutz *et al.* now describe a three-dimensional analysis of the related protein hæmoglobin, at a slightly greater resolution, and show that each of the four sub-units of which this molecule is composed bears a close structural resemblance to myoglobin. It is apparent, therefore, that sperm-whale myoglobin possesses a structure the significance of which extends beyond a particular species and even beyond a particular protein.

We now present the results of a second stage in the analysis of sperm-whale myoglobin; in this the resolution has been increased to 2 Å., that is to say, not far short of atomic resolution. The resulting Fourier synthesis is very complicated, and a detailed study of it will take many months; in the meantime, our preliminary findings may be of interest.

Methods of X-ray Analysis

In this stage we have simply extended the methods which proved successful in the first stage of the analysis, comparing the diffraction pattern of unsub-

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stituted myoglobin crystals with those of the *p*-chloromercuribenzene sulphonate, mercury diammine and aurichloride derivatives, together with a double derivative containing the first two substituents simultaneously. Whereas myoglobin crystals give 400 reflexions having spacings greater than 6 Å., the number of reflexions with spacings greater than 2 Å. is 9,600, each of which has to be measured not only for the unsubstituted protein but also for each of the derivatives. The very much greater number of data posed many problems, both in recording intensities and in computation, and in this stage we relied much more heavily than before on the use of a high-speed computer; it was fortunate that about the time the work began the *Edsac* Mark I computer used previously was superseded by the very much faster and more powerful Mark II.

The data for each derivative were recorded on twenty-two precession photographs; a separate crystal had to be used for each photograph to keep radiation damage within acceptable limits. The results from the different photographs were scaled together on the computer, the best set of scaling factors being determined by solving an appropriate 22×22 matrix[5]. The degree of isomorphism of each derivative was tested, and found adequate, by means of a computer programme which used the *h*0*l* reflexions to refine the preliminary values of the heavy-atom parameters, temperature factor, etc., and then compared the values of $\delta F_{obs.}$ and $\delta F_{calc.}$ as a function of $\sin\theta$. The co-ordinates of the heavy atoms were further refined using correlation functions[6] computed by means of programmes devised by Dr. M. G. Rossmann, and finally refined again during the process of phase determination itself. The phases were determined by essentially the same method as before, but owing to the very large number of reflexions the determination was carried out on the computer rather than graphically. The 'best' phases and amplitudes[7] were computed and used in the final Fourier synthesis, to which a moderate degree of sharpening was applied. In all, some hundreds of hours of computer time were required, and the Fourier synthesis itself, which was calculated at intervals of about 2/3 Å., took about 12 hr.

The Fourier Synthesis

The electron density distribution was plotted in the form of 96 sections perpendicular to x^* and spaced 2/3 Å. apart, the density in each section being represented by a series of contours. For some purposes this method of representation is unsatisfactory, and we have also constructed models of parts of the structure on a scale of 5 cm. = 1 Å., by erecting vertical steel rods parallel to y in an array corresponding to the grid of points in the xz-plane at which densities were calculated, the value of the electron density at points along the rods being indicated by coloured clips. On this scale the whole molecule is about 6 ft. cube, and about 2,500 rods each 6 ft. high are required (see Fig. 4).

The distance between singly bonded carbon atoms is 1·54 Å.; many of the common types of covalent bond present in protein molecules are shorter than this. We could not expect, therefore, that neighbouring covalently bonded atoms would be resolved in the present Fourier synthesis, and in fact such resolution was not achieved; on the other hand, the degree of resolution actually obtained is very nearly

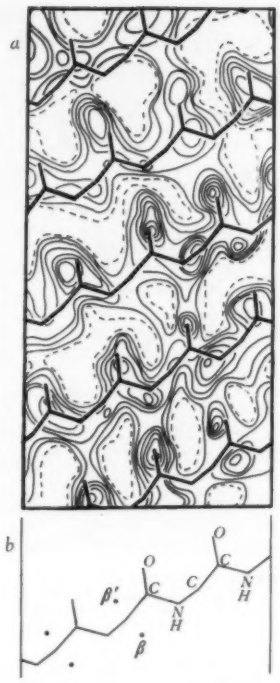


Fig. 1. (*a*) Cylindrical projection of a helical segment of polypeptide chain, with the α-helix structure superposed: for explanation see text. (*b*) Key to the atomic arrangement in the α-helix. The points marked β and β' are the two alternative projected positions of C_β; β is the position in a right-handed and β' that in a left-handed helix of L-amino-acids

as great as theory would indicate, showing that the errors in the synthesis cannot be very large (see Fig. 2). The distances between neighbouring groups in Van der Waals contact is of the order of 3 Å., however, and as would be anticipated, we find that such groups appear as discrete regions of high density. One factor which complicates the interpretation is that similar groups (for example, peptide bonds) in different parts of the structure have substantially different densities. We attribute these differences to variation in the amplitude of thermal vibration in different parts of the molecule. Thus the terminal amino-end of the chain, which is on the outside of the molecule, has low density and is presumably relatively flexible; on the other hand, structures near the hæm group, which is surrounded by several segments of polypeptide chain running in different directions, possess high density, presumably because this part of the molecule is stabilized by many inter-chain bonds.

Configuration of the Polypeptide Chain

In the 6-Å. Fourier synthesis the polypeptide chain appeared as a solid rod of high density with approximately circular cross-section; segments of the chain were more or less straight, and these were joined by dense regions where the chain turned corners. In the 2-Å. synthesis all the straight segments of chain were found to be hollow cylindrical tubes of high density; projecting at intervals from the cylindrical core are dense regions of various shapes and sizes, which are clearly the amino-acid side-chains. More detailed

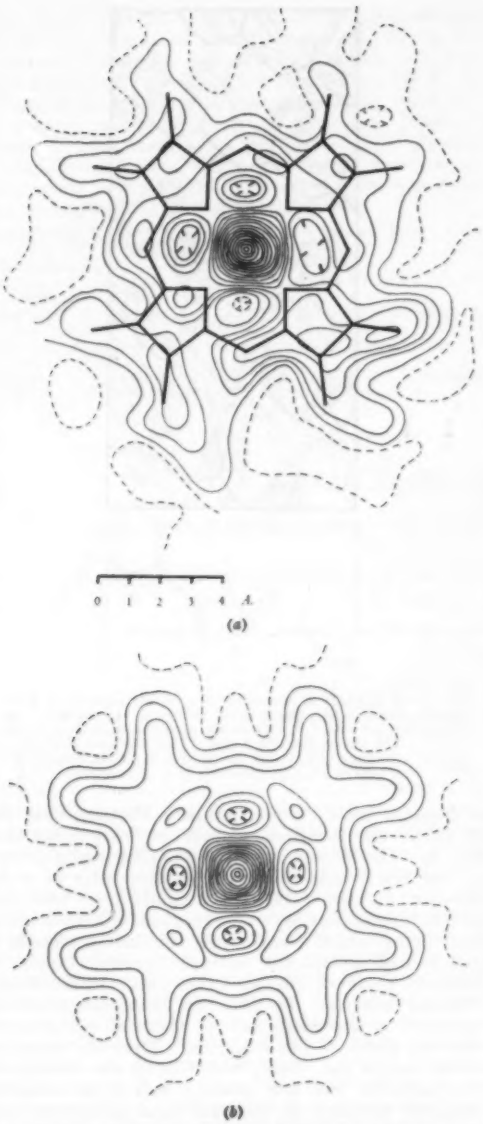


Fig. 2. (*a*) Observed electron density distribution in the plane of the hæm group, with the atomic arrangement in the group superposed. One contour interval = 0·5 electron/Å.³. (*b*) Calculated electron density distribution, computed from the known atomic arrangement as described in the text. One contour interval = 0·5 electron/Å.³

examination shows that, in fact, the cylindrical tubes are helices, consisting of a single strand of high density with axial repeat about 5·4 Å. The density in one such helix is shown in Fig. 1*a*. This was obtained by projecting all the density lying within a cylindrical shell of radii 1·6 and 2·3 Å. on to the surface of a cylinder of radius 1·95 Å. and co-axial with the helix; the cylinder was then cut along a line parallel to its axis and unrolled. Superposed on the contours of electron density is the cylindrical projection of an α-helix, with the dimensions given by Pauling and Corey[8]. It will be seen that the observed density follows the configuration of the α-helix with remarkable precision; and it may confidently be asserted that not only this, but also several other lengths of polypeptide chain which have been analysed in the same way, possess the α-helical configuration. It is also found that the side-chains emerge at intervals of 100° around the periphery of the helix, and at axial intervals of 1·5 Å., in conformity with the parameters of the α-helix. Several indirect lines of evidence had led to the conclusion that parts of the polypeptide chain in globular proteins are in the form of α-helixes, and in hæmoglobin Perutz[9] had found traces of an X-ray reflexion characteristic of this configuration; but these results are the first direct proof that α-helices are present and, indeed, enable them to be seen directly for the first time.

The next question is whether the α-helices are left-handed or right-handed; helices of either hand

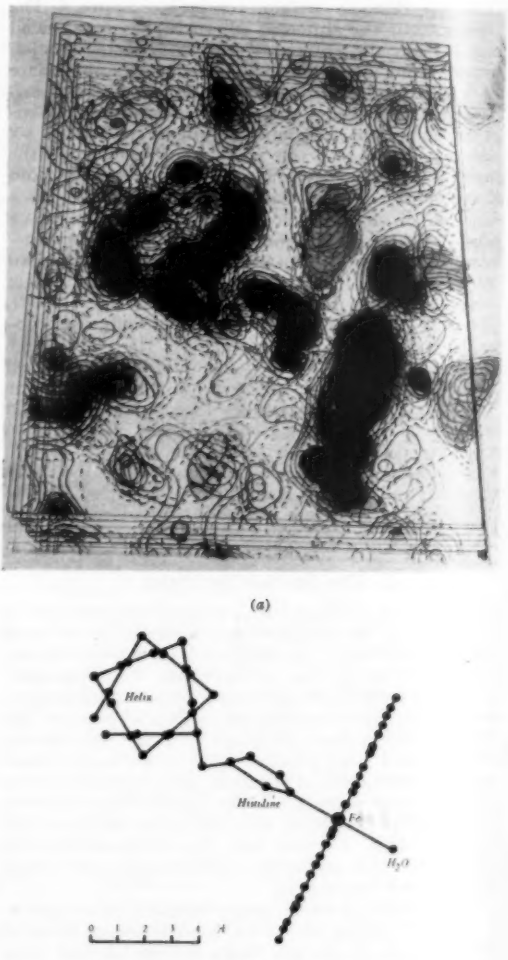


Fig. 3. (*a*) Photograph of a set of sections normal to the plane of the hæm groups showing, from left to right, a helix in cross-section, the histidine residue nearly edge-on, the hæm group edge-on, and a presumed water molecule. (*b*) Sketch showing the atomic arrangement in Fig. 3*a*

can be built, and although the right-handed form appears to be marginally the more stable, the difference is not so great as to make it certain that left-handed helices cannot exist. To decide this question, it is necessary to determine the absolute configuration of the Fourier synthesis; this function has no centre or plane of symmetry and might be plotted in two ways, each of which would be the mirror image of the other. The absolute configuration could be settled by making use of the anomalous dispersion of X-rays from the iron atoms or the introduced heavy atoms; we have not in fact made use of this effect, but instead have proceeded in the following way. In a right-handed α-helix composed of L-amino-acids, the first atom of the side-chain (C_β) projects from the main chain in a direction *opposite* to the C—O bonds of the carbonyl groups; in a left-handed helix of L-amino-acids C_β projects in the *same* direction as the carbonyl groups (see Fig. 1*b*). By constructing a second cylindrical projection (not illustrated) of the density near a radius of 3·34 Å., corresponding approximately to the radius at which C_β is found in either case, we found that C_β was systematically on the side of the main chain opposite the oxygen atoms of the carbonyl groups. This shows that the helix must be right-handed, and we were able to plot the Fourier synthesis with the correct absolute configuration by taking account of the known absolute configuration of a L-amino-acid[10]. The molecule was then found to be of the same hand as all the closely similar sub-units in hæmoglobin, the absolute configuration of which was determined by measurements of anomalous dispersion[4]. All the lengths of α-helix in the myoglobin molecule turn out to be right-handed. Finally, it will be clear from Fig. 1 that it is possible to determine by inspection the direction in which the C—O group points, and hence to see which is the terminal carboxyl end of each segment.

The α-helices can also be located by building atomic models into the model of the unit cell made with steel rods; this has been done for all the segments of helix in the molecule, and it is found that the total number of amino-acid residues contained in these segments is 100–110, whereas the number of residues contained in the whole molecule is believed to be 153 (Edmundson, A., unpublished results). Thus, 65–72 per cent of the molecule consists of regular right-handed α-helix, made up of about eight segments, each containing between seven and about twenty residues.

When the chain turns a corner, its regular helical configuration is necessarily disrupted. At the present resolution the precise arrangement of the residues at corners is difficult to determine; we have, nevertheless, built plausible models of several corners, though we do not yet claim to have established their configurations with certainty. Most of the corners take up two or three residues, and in addition there is one region (on the extreme right of Fig. 5*a*), consisting of about 13–18 residues, in which the arrangement is irregular (part of it is helical, but probably not α-helical). Further studies will be devoted to elucidating these problems.

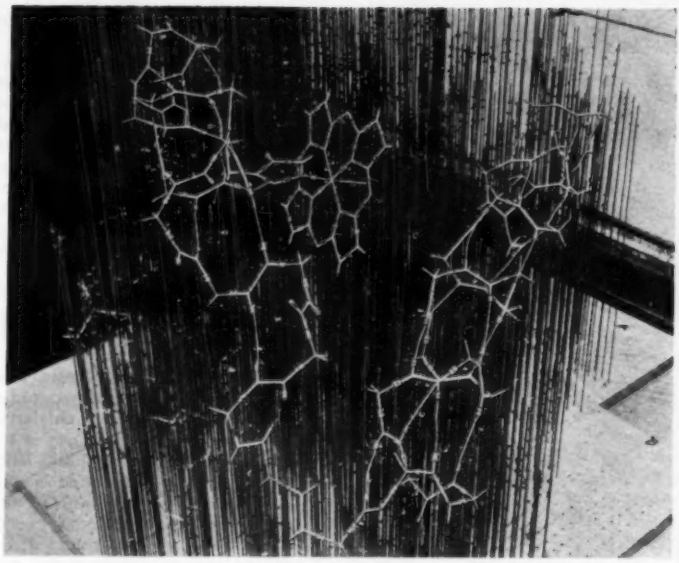
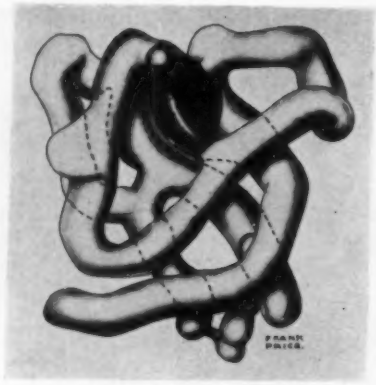


Fig. 4. Photograph of a model of part of the molecule near the hæm group, showing the vertical rods and coloured clips which indicate the electron density at each point of the grid, and atomic models of the hæm group and neighbouring helices. The terminal carboxyl end of the chain is on the extreme left

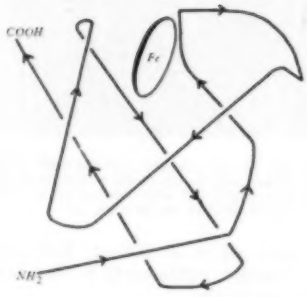
No serious attempt has yet been made to identify the side-chains, and indeed it is doubtful whether this can be done systematically at the present resolution, partly because many of them appear to be subject to considerable thermal vibration, partly because there are often interactions between two side-chains on adjacent turns of the same helix or on adjacent helices, and it is difficult to tell where one ends and the other begins. In special cases, however, identification is easy; thus, in several places two helices approach one another closely, and one or more side-chains must for steric reasons be glycine. On the other hand, some side-chains are so long that they can only be arginine or lysine. It remains to be seen how many positive identifications can be made; but it is clear that if the whole amino-acid sequence of the protein were known, it would be possible at this stage to construct a model of the complete structure with fair precision. Unfortunately, this information is not yet available; but in the meantime it may be possible to correlate and check our findings with the partial results of the sequence determination now being undertaken by Mr. A. Edmundson, of the Rockefeller Institute.

The Hæm Group

The identification of a disk of high electron density in the 6-Å. synthesis as the hæm group is confirmed by the 2-Å. synthesis; but the iron atom is now just resolved from the nitrogens of the porphyrin ring (the iron–nitrogen distance is about 1·9 Å.) and the structure of the group as a whole corresponds closely with theoretical expectation. Fig. 2*a* shows a section through the density distribution, cut in the plane of the hæm group; superposed on the section is a model of the hæm group with the dimensions



(a)



(b)

Fig. 5. (a) Drawing of the tertiary structure of myoglobin as deduced from the 6-Å. Fourier synthesis. (Note: the plane of the hæm group is indicated incorrectly in this drawing—see text). (b) The course of the polypeptide chain, deduced from the 2-Å. Fourier synthesis

found by Crute in his recent structure analysis of nickel etioporphyrin[11]. In the model, only the carbon atoms of the methyl groups and the first carbon atoms of the vinyl and propionic acid groups have been indicated; our analysis has not yet proceeded far enough to identify the latter groups. Fig. 2*b* is a Fourier synthesis made by calculating structure factors for the model structure in a convenient unit cell, applying to these the same temperature factor as that observed in myoglobin, and using them as terms of a Fourier synthesis cut off at 2-Å. spacings. It will be seen that the calculated and observed density distributions agree very closely; furthermore, the observed peak density (8·8 electrons/Å.^{3}) and half-width (1·8 Å.) of the iron atom agree well with the predicted values (8·2 electrons/Å.^{3} peak density, 1·8 Å. half-width). Errors are, of course, inherent in a Fourier synthesis produced from experimental results; the good agreement between the observed and calculated versions of the hæm group, and between the helical polypeptide chain and the α-helix (Fig. 1), indicate that in the present case these errors are fairly small, certainly less than we had anticipated.

In one respect the 6-Å. model now requires correction. Measurements of electron spin resonance[12] had shown that the two hæm groups in the unit cell were tilted out of the *bc* plane through 21° about an axis parallel to *c*, one in one direction and one in the other, but from these measurements one could not decide which direction of tilt was associated with the hæm group of which molecule. In the 6-Å. analysis the wrong choice was made; the new results show that the angle of tilt is indeed very close to 21°, but that it should be in the direction opposite to that shown in earlier models (including Fig. 5*a*).

Chemical studies of hæmoglobin and myoglobin have suggested that the hæm group is attached to the protein by a bond from the iron atom to a nitrogen atom of a histidine residue, and that the sixth co-ordination position of the iron atom is occupied in met-myoglobin (at *p*H's below 7) by a water molecule. Our results are in conformity with these suggestions. Fig. 3*a* is a photograph of a series of sections cut through the hæm group normal to its plane. The hæm group is seen edge-on; to its left is the histidine residue, also nearly edge-on, and to the left of that is the helix to which the histidine is attached, seen in cross-section. To the right of the hæm group is a small isolated peak which we take to be the oxygen atom of the water molecule (see Fig. 3*b*). When this structure is built with atomic models (see Fig. 4), it is found to satisfy all known requirements as to bond-lengths, angles, etc. Thus the iron–oxygen distance is close to 2·1 Å.; that between the iron and one of the histidine nitrogen atoms is 1·9 Å., while the second histidine nitrogen points with its hydrogen directly at a carbonyl group on the helix, presumably forming a hydrogen bond (forked at the carbonyl end); and finally the bonds about the C_α—C_β bond of histidine are almost exactly in the staggered configuration.

General Configuration of the Molecule

In the 2-Å. synthesis the continuity of the polypeptide chain can be followed throughout the molecule; in addition, its direction can be ascertained in each straight segment by examining the direction of the carbonyl groups in the cylindrical projections or by direct model-building. The 'run' of the chain so deduced everywhere agrees with that derived from the 6-Å. synthesis, as well as resolving several ambiguities in that model, and the terminal amino- and carboxyl-ends of the chain can now be identified. The arrangement is shown in Fig. 5 (see also Fig. 5 in the accompanying article by Perutz *et al.*). The overall shape of the molecule is as previously deduced.

Conservative estimates suggest that it may now be possible to locate with some precision at least 43 per cent of all the atoms (excluding hydrogens) in the molecule—namely, those making up the hæm group and the backbone of the regular segments of helix, together with the associated C_β's. We believe that it may be possible, either by straightforward refinement procedures or by heavy-atom methods, to extend the resolution of the synthesis to a point near the limit of the diffraction pattern of sperm-whale myoglobin crystals (about 1·5 Å.), and thus to reveal in any event most of the details of the atomic arrangement of the molecule. Further work is in hand with this end in view.

We wish to thank the Rockefeller Foundation for its generous financial support; the National Science Foundation (U.S.A.) for providing a fellowship for one of us (R. E. D.); to the National Institutes of Health (U.S.A.) for fellowships (R. G. H., R. E. D.); and to the Director and Staff of the Mathematics Laboratory, University of Cambridge, for facilities on the *Edsac* and for their enthusiastic co-operation. We also wish to thank Miss Mary Pinkerton, who was responsible for the organization of the very

extensive programme of data processing, and who helped us in many other ways; a large number of computing assistants, especially Mrs. J. Blows, Mrs. W. Browne, Mrs. V. Cotterill, Mrs. A. Hartley, Miss J. Hughes, Mrs. J. Moss, Mr. P. Mulhall, Mrs. K. Parkes and Miss E. Rowland; Dr. M. G. Rossmann and Dr. E. Alver for making available several of their computer programmes; and Sir Lawrence Bragg for his encouragement at all stages of the work.

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DEVELOPMENTS IN GAMMA-RAY OPTICS

By Prof. P. B. MOON, F.R.S.
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DURING the past decade, several phenomena that had previously been observed with light or with X-rays have been studied with nuclear γ-rays, where the quantum energy is typically 100 keV.–10 MeV. and the wave-length correspondingly short, 10^{-9} to 10^{-11} cm. Some new phenomena, specifically nuclear or specifically high-energy, have been added: but most of the experiments, though often technically more difficult, are extensions of those familiar at lower energies.

An excellent example of such an extension is the curved-crystal γ-ray spectroscopy of γ-rays, achieved with great elegance and precision by DuMond[1] and his collaborators. It will suffice to quote their value for the wave-length of electron–positron annihilation radiation, namely, $24{\cdot}262 \pm 0{\cdot}0033$ mÅ., and to mention that this radiation, when generated within a metal, has been observed to possess a spectral width which reflects the spread of energy of the conduction electrons.

A second example is the observation in the γ-ray region of Rayleigh scattering, a name taken over from visual optics to describe scattering of photons by the electronic structure of an atom without change of the atom's quantum state and therefore without any change of the photon's energy, except the small fraction taken by the recoil of the atom necessary to conserve momentum. This is the 'unmodified line' of X-ray scattering terminology, as opposed to the 'modified line' that arises from Compton scattering. The existence and (if it existed) the origin of elastic scattering at higher energies was a matter of controversy in 1930–33, but it is now clear that Meitner and Kösters observed it[2] and that its origin was electronic, not nuclear. In taking up the study of this scattering process with the wider range of monoenergetic lines provided by artificial radionuclides, Moon[3], Storruste[4] and Wilson[5] were able to verify semi-quantitatively the calculations of Franz[6] regarding its intensity and angular distribution, and many other workers have since made accurate experiments at energies above 1 MeV. where Franz's theory (which is essentially a Debye form factor) must be replaced by the more accurate relativistic treatment of Brown, Peierls and Woodward[7].

Two phenomena not observable in the X-ray region were predicted to be interwoven with the Rayleigh scattering; first, the classically calculable 'Thomson' scattering of photons by the nucleus, which oscillates in the electric field of the incident wave and radiates at the same frequency; secondly, scattering by virtual pair production in the field of the nucleus, which is a specifically quantum phenomenon.

The interest in the nuclear Thomson scattering seems to date from 1950, when it was remarked[8] that it should be coherent with Rayleigh scattering, but the process must surely have been considered long before then; the virtual pair production process was suggested as long ago as 1933 by Delbrück in a note to the paper of Meitner and Kösters[2].

Both processes were difficult to identify because the scattering radiation is of exactly the same frequency as the Rayleigh scattering but, at ordinary γ-ray energies, of much smaller intensity. The Thomson scattering was plausibly identified by the fact that the observed angular distribution of the total elastic scattering agreed much better with theory when this component was added into the calculation; its existence and its interference with the Rayleigh component were positively established by Sood's[9] measurements of the polarization of the elastic scattering at about 1 MeV., at which energy the Rayleigh and nuclear Thomson components have substantially different polarizations. The use of polarization for examining elastic scattering of γ-rays is pleasingly reminiscent of Lord Rayleigh and the blue of the sky.

The characteristic features of the Delbrück process are that its magnitude increases as a high power of the quantum energy and its angular distribution is strongly peaked around 0°. It has at last been unquestionably observed by Moffatt and Stringfellow[9] in Oxford, using 90-MeV. radiation from an electron synchrotron and working at angles of about 0·1°.

Another process of elastic scattering of γ-rays is that associated with the excitation of a nucleus from its ground-state to an excited state followed by the re-emission of a photon. This is the nuclear analogue of the optical resonance radiation so beautifully demonstrated by R. W. Wood in the early years of this century. It was sought experimentally at intervals over twenty years, the difficulty being not that the process is intrinsically weak (its cross-section, like that of any comparable resonant process, can be of the order of $\lambda^2/4\pi$) but that γ-ray lines are so narrow that the loss of energy to nuclear recoil ($h^2\nu^2/2Mc^2$ on emission and equal amount on absorption) destroys the resonance[10].

It was observed ten years ago[10] by the use of a high-speed rotor (not an ultracentrifuge, as it is called by some writers; the correct term is probably 'motor', since it is motion, not specifically rotary motion, and certainly not centrifugal force, that is needed) on which the source of γ-rays was mounted, so that the Doppler effect of bulk motion of the sources brought the emission spectrum into coincidence with the resonance of the scatterer. Both the emission and the absorption lines are broadened far beyond their natural width by the Doppler effect of thermal motion. Experimental results obtained by Davey and Moon[11] demonstrated the essentially thermal shape of the mercury-198 resonance line; its width is less than 1 eV. in 411 keV., which is comparable with the fractional width of the narrowest visible lines.

The phenomenon has been observed with several means of securing partial overlap of absorption and emission lines: by heating the source[12], by using atomic recoil from a preceding beta, gamma or neutrino emission[13–15] or by absorption of the resonance radiation in a second layer of the same material[14]. It has proved valuable for measuring the life-times of nuclear levels in the range 10^{-10} to 10^{-14} sec., and details such as the angular distribution[16] and the plane polarization[17] of the resonance radiation at 90° have been duly verified.

When the excited state decays largely through an intermediate level, the fluorescent radiation can be observed[18]. A particularly interesting application of the neutrino-recoil method was made by Goldhaber, Grodzins and Sunyar[13], who showed that in special circumstances the resonance radiation can be circularly polarized; they used this fact to determine the spin polarization (helicity) of the neutrino.

One is not restricted to γ-rays from radioactive sources: nuclear reactions, with the forward momentum of the excited nucleus as the overlapping agent, have been used both in scattering[18] and in absorption[19,20] experiments, and bremsstrahlung has been used to excite a particularly broad level[21].

A major development was reported in 1958 by Mössbauer in Munich[22]. He showed, using the 121-keV. line of iridium-191, that in crystals at low temperatures appreciable fractions of the emission and resonant absorption processes for low-energy γ-rays occur without nuclear recoil and indeed with no loss of energy between the γ-ray and the crystal other than that due to the recoil of the crystal as a whole; for these fractions, the line has only its natural width and may be brought out of resonance by relative motion of source and absorber of the order of 1 cm./sec. as compared with the order of 10^4 cm./sec. required to sweep the thermally broadened components over one another. The Mössbauer line sits as an extraordinarily tall and narrow spike on top of the thermal profile, at the same position for both emission and absorption. The cross-section for the Mössbauer effect is a sizable fraction of its ideal value ($\sim \lambda^2/4\pi$) and, even at 100 keV., can be appreciable in comparison not merely with the Rayleigh but also with the Compton and photoelectric cross-sections.

It is a curious reflexion on the journal-reading habits of physicists that Mössbauer's discovery was not generally known for a year after its publication; during the past few months, workers in many laboratories have realized its exciting possibilities. These are of two classes: its use to investigate the response of crystals to the offer of amounts of energy comparable with $k\theta$, where k is Boltzmann's constant and θ the Debye temperature, and the much more varied class of experiments that exploit the extreme narrowness of the line.

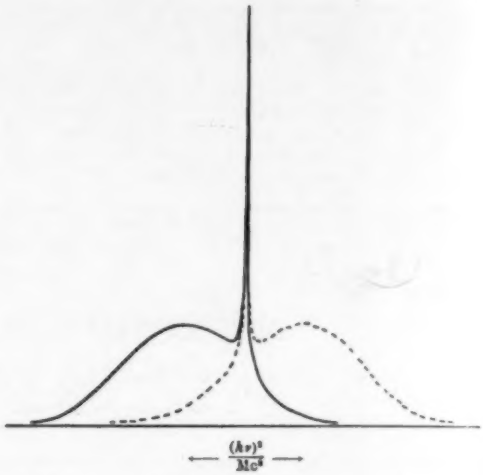

Fig. 1. Emission line profile (full line) and absorption line profile (broken line). The narrow line is present only with low γ-ray energies and cold crystals, when it may be $\sim 10^6$ times as high as the surrounding thermally broadened line and contain a large fraction of the total area

The effect has recently been observed in several laboratories with the 14 keV. line in iron-57, which has a width of the order of 10^{-8} eV.—one part in 10^{12} of its energy. It is, in fact, extremely easy to demonstrate, requiring only a thin source of cyclotron-irradiated iron, an absorber of ordinary iron, and a proportional counter or scintillation counter. Even at room temperature, changes of counting rate of the order of 30 per cent are observed when the source is oscillated at a speed of less than a millimetre per sec.

Perhaps the most exciting application of the Mössbauer effect is to observe the effect of gravitational fall on the energy of the photons (the 'terrestrial red-shift experiment') which appears measurable over a vertical distance of less than 100 m.: such experiments are understood to be in progress in the United States, England (Harwell and Manchester)[24] and the U.S.S.R. Another interesting experiment, being tried at Harwell and Birmingham, relates to special rather than general relativity; the source is mounted at the centre of a spinning rotor, the resonant absorber at the edge so that, though the distance between them does not change, the photons meet an absorber travelling exactly at right angles to their direction of flight: a second-order Doppler effect ($\frac{1}{2}v^2/c^2$) should be observable.

Mössbauer lines will have structures attributable to the interaction of the crystal's intrinsic magnetic or electric field with the magnetic dipole or electric quadrupole moments of the ground and excited states of the nucleus; Pound and Rebka[24] have already observed the splitting of the iron-57 line in the intrinsic magnetic field of iron. This is distinct from, and much narrower than, the structures due to the excitation of phonons in the crystal which, as Mössbauer pointed out, are analogous to effects observed in the

scattering of very slow neutrons in crystals. In favourable instances the nuclear Zeeman effect should be observable.

One may imagine, too, searches for effects of electric or magnetic fields upon any small electric or magnetic moments that may be possessed by free photons, and one can see interesting problems concerning the limitations to the possible narrowness of Mössbauer lines from transitions still longer-lived than the 10^{-7} sec. iron-57. Are these limits due to fluctuations in crystal fields, to the finite size of the crystal (or of whatever part of it may act as a unit in declining to accept the proferred recoil energy) or to other causes? If the relative motion of the source and the absorber is at a frequency high compared with the inverse lifetime of the excited state, will the process recognize or ignore this motion?

The recoilless absorption process should be accompanied by sharply resonant scattering, weakened by the strong internal conversion of low-energy gamma transitions. In experiments at Birmingham, with gold-197, it appears just observable. This scattering should be coherent with the Rayleigh component and, in favourable circumstances, should show the lattice interference patterns familiar in X-ray work from which not only the positions of the resonantly scattering nuclei but also the phase of scattering should be measurable.

Thus, after a long period of little progress, γ-ray optics now offers a range of phenomena comparable with those known in the fields of light and of X-rays, and, though admittedly in rather special circumstances, a far superior resolving power.

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OBITUARIES

Prof. P. Lebeau

PAUL LEBEAU, professor of pharmaceutical chemistry, Paris, and member of the Paris Academy of Sciences, died on November 18, having nearly reached the age of ninety-one.

Lebeau began his career in chemistry as a student at the École de Physique et de Chimie, Paris, and his outstanding qualities were immediately appreciated by Prof. Étard. His early publications attracted the attention of H. Moissan, and when the latter was appointed to the chair of chemistry in the Faculté des Sciences at Paris, Lebeau was made director of his laboratory. His researches in inorganic chemistry, particularly with regard to fluorspar and fluorine, led to the degree of D.Sc. in 1898. During this period, he was also studying to become a pharmacist, and he achieved this object in 1899.

Lebeau was appointed to the chair of toxicology in 1908, which position he held until 1918. He deliberately orientated this subject towards industrial hygiene and became intensely interested in its medico-legal aspects. As a result, his services during the First World War were of outstanding merit, and in 1918 he succeeded Prof. Charles Moureu in the Berthelot chair in the Collège de France. His great energy and outstanding ability were then directed towards the subject of pharmaceutical chemistry, and his efforts met with similar international recognition as they had already done in the realm of toxicology. His treatise on pharmaceutical chemistry has passed through many editions and is accepted as a standard work on this subject.

As president of the Commission des Hautes Températures of the Centre National de la Recherche Scientifique he was largely responsible for the outstanding success of this body, which resulted in the publication in 1924 of an important book entitled "Fours Électriques et Chimie". This work has attained international recognition.

Not unnaturally, the services of a scientist of such outstanding merit were in great demand, particularly in connexion with government and international committees. These services were given without restraint, and Lebeau made outstanding contributions during his connexion with the Commission des Études Chimique de Guerre, the Conseil d'Administration du Centre National de la Recherche Scientifique, the Commission Internationale des Poids Atomique, and the Société Chimique de France.

Prof. Lebeau's long life covered the period when the science of chemistry was expanding rapidly, a period which was characterized by the introduction of many new chemical concepts. He played his part in this new thinking and can be regarded as one of the architects of modern pharmaceutical chemistry. His career and devotion to the science should be a stimulant to all future students of chemistry.

W. H. LINNELL

Mr. P. R. Coursey

MR. PHILIP RAY COURSEY, of the Dubilier Condenser Co., Ltd., died on January 3. Mr. Coursey, who was born in 1892, was educated at University College, London, and became assistant to Sir Ambrose Fleming at the College. During the First World War he was Admiralty inspector of wireless telegraphy in H.M. Auxiliary Patrol, and in 1919 became technical research assistant at H.M. Signals School. During

1920–23 he was on the editorial staff of the *Radio Review* and the *Wireless World*, to the second of which journals he was a frequent contributor. During this period he was an active and enthusiastic member of the Wireless Society of London (later, the Radio Society of Great Britain); and he was responsible for organizing the British amateurs who in 1921 co-operated in the tests conducted by the American Radio Relay League for establishing radio communication between the United States and Britain. Mr. Coursey was also an active experimenter in the early development of wireless telephony, and was the author of several books on this and allied subjects. He became chief engineer of the Dubilier Condenser Co., Ltd., in 1923, a position which he held until 1931, when he was appointed technical director of the Company; he retained that position with distinction until his retirement in 1957, when he was appointed technical consultant in addition to retaining his directorship.

Mr. Coursey was the originator of many important developments and held numerous patents. His extensive knowledge and experience have been a valuable contribution to the industries with which he was connected. He also took a prominent part in and made valuable contributions to the deliberations of the electrical and telecommunications industries both on British and international committees.

NEWS and VIEWS

Theoretical Physics at St. Andrews : Prof. R. B. Dingle

Dr. Robert Balson Dingle, reader in theoretical physics in the University of Western Australia, has been appointed as the first occupant of the new chair of theoretical physics in St. Salvator's College of the University of St. Andrews. After a notable undergraduate career as Bournemouth Borough Scholar and major scholar at St. John's College, Cambridge, during which he held the Wright Prize twice and also the Hughes and Hockin Prizes, and at the end of which in 1946 he confused his Tripos examiners by, in effect, dismissing their questions and setting and answering his own, he found a corner in the Royal Society Mond Laboratory and began his research career, shortly thereafter being appointed to a fellowship at his College. Almost immediately, he began to publish, and a steady stream of valuable papers has continued since, uninterrupted by a series of moves of increasing geographical amplitude, first to Bristol, then Delft, then Ottawa, and then Nedlands in Western Australia.

Dr. Dingle's publications have covered three main fields and a few small ones. First, chronologically, came a series of elegant papers clarifying the hydrodynamics of liquid helium II, and in particular, second sound. This was followed by a series on collective electron effects in metals, in which diamagnetic or cyclotron resonance was predicted. This series also contained several important contributions to the understanding of the complicated oscillatory magnetic phenomena in metals known as the de Haas–van Alphen effect, and the removal of the apparent discrepancy between observation and theory of optical reflectivity in metal, by an extension and correction of the Reuter–Sondheimer theory. The most recent series of papers is more mathematical and is concerned with the evaluation of integrals used in mathematical physics. In addition to these three main streams, he has also made useful contributions on semi-conductors and on quantum and statistical mechanics.

Pharmacology in London : Prof. G. V. R. Born

Dr. G. V. R. Born, recently appointed to the Vandervell chair of pharmacology at the Institute of Basic Medical Sciences in the University of London, took his M.B. and Ch.B. at Edinburgh in 1943 and his D.Phil.(Oxon) in 1951. After his return from service as army pathologist in the Far East, he took up research in the rapidly developing new field of biochemical pharmacology. While on the staff of the Medical Research Council's Toxicology Research Unit he became interested in metabolic inhibitors, and, on returning to the Nuffield Institute for Medical Research at Oxford in 1953, he studied the actions of these inhibitors on smooth muscle. His main field of work has been on the pharmacologically active amines, and this led him to study the blood platelets by biochemical and pharmacological methods. A most notable contribution was the discovery of large amounts of adenosine triphosphate in platelets. The role of this substance in the uptake and storage of 5-hydroxytryptamine by platelets and its fate during clotting are now being explored. Dr. Born has also taken an active part in the teaching at Oxford, both in the Departments of Pharmacology and Pathology, and as a medical tutor at St. Peter's Hall. He has travelled widely and is well known to his scientific colleagues on both sides of the Atlantic.

College of Advanced Technology, Birmingham : Dr. T. Lupton

Dr. T. Lupton has been appointed head of the Department of Industrial Administration of the College of Advanced Technology, Birmingham, as from April 1. At present senior lecturer in industrial sociology in the University of Manchester, Dr. Lupton began his career as an apprentice marine engineer, and later became an engineering draughtsman. He afterwards gained a diploma in economics and political science at Ruskin College, Oxford, proceeded to an honours degree in economics at Oriel College, Oxford, and was awarded the degree of Ph.D. at Manchester in 1959. Dr. Lupton has carried out a number of investigations on the relationship between occupation and social status, and recently the Department of Scientific and Industrial Research granted him £29,000 for a five-year research project on "Social Factors affecting Workshop Behaviour"; he will be continuing this work at Birmingham with the aid of Research Fellows and assistants.

National Institute of Agricultural Botany : Mr. G. W. G. Briggs

Mr. G. W. G. Briggs has been appointed assistant director of the National Institute of Agricultural Botany. Since 1948 he has been head of the Seed Production Branch of the Institute, where he was responsible for the development of field approval and

certification schemes for the improvement of cereal, herbage and vegetable seeds. The post of assistant director was created following the resignation of Mr. A. Sandison, who has been deputy director of the Institute during the past seven years.

Institution of Electrical Engineers: New Honorary Members

THE Council of the Institution of Electrical Engineers has elected the following to honorary membership: Colonel B. H. Leeson, recently director of the British Electrical and Allied Manufacturers' Association, in recognition of his extensive contributions to the application of electrical science and engineering in the manufacture of electrical plant and equipment, to the development of co-operative effort within the electrical industry and to the training of electrical engineers, and of the services he has rendered to the Institution during nearly half a century; and Dr. Richard L. Hearn, for his notable contributions in the development of electricity generation and its supply for the benefit and use of mankind, particularly in Canada, and for the part he has played in international co-operation in this field. Dr. Hearn was elected under the provisions of the rule whereby the Council is empowered to elect, once in every three years, an additional honorary member who is not at the time of his election a member of any class of the Institution.

Faraday Medal

THE thirty-eighth award of the Faraday Medal of the Institution has been made to Sir George Thomson, president of the British Association, in recognition of his outstanding contribution to the advancement of electrical science, of the leadership in research which he has given to others in this field, and of his inspiring services to education in science and technology. This Medal is awarded not more frequently than once a year, either for notable scientific or industrial achievement in electrical engineering or for conspicuous service rendered to the advancement of electrical science, without restriction as regards nationality, country of residence, or membership of the Institution.

Youth Services in Great Britain

IN the House of Commons on February 3, the Minister of Education, Sir David Eccles, announced that the Government accepted in principle all the main recommendations for Government action of the report of the Committee appointed in November 1958 under the chairmanship of Lady Albemarle to review the contribution which the youth service in England and Wales could make in assisting young people to play their part in the community. The necessary discussions between the Ministry of Education, the local education authorities and the voluntary youth organizations, with the view of taking precise decisions how to act on the Albemarle recommendations, will take place urgently. The Government has decided that deficiencies in voluntary and paid workers, in buildings and facilities for sport, in money and enthusiasm, must be made good, though everything cannot be done at once, and the will to succeed must come from the local areas at least as much as from the centre. The Government will finance by Exchequer grant most of the recommended expansion in training facilities from 700 to 1,300 full-time youth leaders by 1966, and Sir David hopes an emergency training college will be open by January 1, 1961. He proposes to ask interested parties to examine with him the execution of the recommendation regarding salaries and opportunities for transfer to other careers. Grants for starting buildings by the local authorities and voluntary organizations in the next two years will be authorized up to £2 million, and grants to voluntary projects will be increased. Grants will be increased for headquarters expenditure of voluntary organizations for approved experiments in attracting young people not now using the youth service. Sir David said that, to begin with, he proposes himself to take the chair of the recommended Youth Service Development Council.

Sir David undertook to consider a suggestion that the House should discuss the Albemarle and the Crowther Reports together, and said that the Government proposes to accept the recommendation that the age-range of the youth service should be 14–20 years. A corresponding statement was made on the same day by the Minister for Science, Lord Hailsham, in the House of Lords.

Gasification of Coal and Oil

REPLYING to a question in the House of Commons on February 1, the Minister of Power, Mr. R. Wood, said the Gas Council is spending £120,000 this year on research into coal gasification, and the National Coal Board and the Central Electricity Generating Board £680,000 and £130,000, respectively, on coal utilization. The Gas Council is spending about £230,000 on research into the gasification of oil under a programme intended to contribute also to the improvement of the economics of coal gasification, and another £240,000 on research into problems of gas purification and the treatment of by-products.

Trans-Pacific Telephone Calls

THE United Kingdom Government has accepted the report of the Pacific Cable Conference, which was held in Sydney during September–October 1959, and in which Government representatives from Canada, Australia, New Zealand and the United Kingdom participated. Cable and Wireless, Ltd., will act for the United Kingdom Government in the construction, maintenance and financing of the cable. The total estimated cost is £26·3 million, of which the United Kingdom share is £8·3 million. The cable will be more than 8,000 nautical miles long and will include more than three hundred under-sea amplifiers. In places, cable and amplifier will be laid to a depth of almost four miles. The project is to be undertaken by a joint Commonwealth Management Committee. The Committee will be under the leadership of Mr. R. J. Halsey, director of research of the United Kingdom Post Office. A light-weight cable will be used with rigid repeaters developed in the United Kingdom and being used in Cantat, the cable between the United Kingdom and Canada. The Trans-Pacific Cable will have a capacity for at least eighty simultaneous conversations. Any one of the telephone circuits can be used to provide twenty-four telegraph channels.

National Central Library

THE forty-third annual report of the Executive Committee of the National Central Library, covering the year ended February 28, 1959, records that although the requests for loans continue to become more specialized and inaccessible in nature, the proportion of applications handled successfully continues to rise and is now 76 per cent (National Central

Library. 43rd Annual Report of the Executive Committee for the year ending February 28, 1959. Pp. 20. London: National Central Library, 1959). Attention has been given to the problem of increasing the speed with which books can be obtained, and here delays are chiefly due to the failure of some libraries to deal promptly with the requests of the National Central Library. The Executive Committee appeals strongly to all co-operating libraries and regional bureaux to help by dealing with requests with the minimum of delay. Total applications again decreased, from 79,971 in 1957–58 to 77,004, and of the 76,048 volumes issued from or through the Library (including renewals), 32,666 were to or from public libraries; 12,202 to or from university libraries; special outlier libraries, 8,704; overseas libraries, 3,077; Government departments, 2,528; industrial and research organizations, 3,497; and adult classes, 11,286. Overseas libraries lent 1,804 volumes and borrowed 2,488 volumes, and 240 and 432 further requests, respectively, were satisfied by photographic reproductions. The outlier libraries, now numbering 281, lent 14,525 volumes, and other special libraries a further 2,527 volumes. Regional co-operation covered 163,842 loans within systems, and 22,099 outside systems and the university libraries supplied 24,390 books and borrowed 12,202. The arrears in the incorporation of entries for the union catalogues increased and the problem has been specially examined. There was a marked increase in the work of the British National Book Centre.

Teacher Training in Britain

More than 31,000 men and women are now training to become teachers, an increase of nearly a third compared with the 24,500 under training five years ago. The number entering training colleges this autumn had also increased by a third—16,000 compared with 12,000 in 1955–56. Standards are rising, most of the students now having more than the minimum academic qualifications for entry. The training colleges will thus be sending a record number of trained teachers into the schools in 1961. This will help to tide over the so-called 'year of intermission' in 1962, when there will be fewer newly trained teachers entering the schools as a result of the lengthening of the training course from two to three years. Expansion of the teacher training colleges by nearly two-thirds is now under way; many of the 130 training colleges are to be extended and new colleges built.

Recruitment for next year's intake is already starting, and the Ministry of Education has issued a revised booklet, "Becoming a Teacher", to help young people who want to teach in primary or secondary schools. The booklet describes the work the teachers do, the schools in which they teach, the qualifications they require and the training they undertake. It includes information about the training grants available and the salaries paid to teachers. The booklet can be obtained free from any local authority or from the Ministry of Education, Curzon Street, London, W.1.

Rubber Industry Display at the Science Museum

A permanent display representative of both producing and manufacturing sides of the British rubber industry has been presented to the Science Museum, London. It occupies three large show-cases in the industrial chemistry gallery, and illustrates the molecular structure of natural and synthetic rubbers; their production, compounding, and processing; the great variety of products thus obtained, and the vital part they play in modern life. The display has been devised and organized by the Federation of British Rubber and Allied Manufacturers and made possible by the generosity of British manufacturers of rubber products; makers of synthetic rubbers, compounding chemicals, processing machinery and components; the Natural Rubber Development Board, representing producers of natural rubber; and the National College of Rubber Technology.

National Museum, Bloemfontein

The annual report of the National Museum at Bloemfontein for the year ended March 1959 records the installation of a model of a gold mine and a dioramic display of a Bushman's cave showing rock paintings, stone implements and two modelled figures of men, one in the act of painting against the rock wall, while the other is making stone implements. In contrast with other museums, it was found that an admission charge curtailed severely the number of visitors, and after an experimental period of six months the charge was withdrawn. Much research work by the staff is in progress or has been completed. This includes studies on fossil hippopotami, engraved Bushmen sticks and Karoo vertebrate fossils.

Collecting Fossils, Minerals and Rocks

The seventh edition of "Instructions for Collectors", No. 11, published by the British Museum (Natural History) at 1*s.*, follows the usual lines of this well-known and excellent booklet. It will be found to be of use to all types of collectors as, in addition to very elementary instructions, details are given of preparations which have only recently been discovered. The new materials mentioned have in all cases been tested extensively and may be used with confidence.

Marconi International Award

The establishment of an annual award in memory of Guglielmo Marconi is announced by Permindex, the World Trade Centre in Rome. This award will be made to the person making the greatest contribution to the development of Marconi's discoveries. Recipients will be chosen by a committee composed of important international personalities, a member of the Marconi family and representatives of Permindex. This announcement followed the dedication of the Marconi obelisk, to be known as the Marconi Antenna, in the Piazza Italia around which are grouped the four majestic buildings shortly to be occupied by Permindex. To celebrate the occasion the Square itself was renamed Piazza Guglielmo Marconi. The prize consists of a small reproduction in gold of the obelisk. Further information can be obtained from Industrial Trade Fairs, Ltd., Drury House, Russell Street, Drury Lane, London, W.C.2.

Ramsay Memorial Fellowships in Chemistry

The Trustees of the Ramsay Memorial Fellowships Trust will consider in June applications for two fellowships for advanced students of chemistry. One of the fellowships will be limited to candidates educated in Glasgow, who can apply to be considered for either fellowship. The value of each fellowship will be £600 per annum, to which may be added a grant for expenses of research not exceeding £100 per annum. The fellowships will normally be tenable for two years. Further information can be

obtained from the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London, W.C.1. Completed application forms must be received not later than April 11, 1960.

Lady Tata Research Scholarships and Fellowships

THE Trustees of the Lady Tata Memorial Trust invite applications for grants, scholarships and fellowships for research on diseases of the blood, with special reference to leukæmia, in the academic year beginning on October 1. In view of the affinity between leukæmia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukæmia will be eligible for consideration. Further information can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3. Applications must be submitted before March 31, 1960.

Lectures at the Imperial College, London

THE nine inaugural lectures delivered at the Imperial College of Science and Technology during 1956–57 and 1957–58 have now been issued as a bound volume. They comprise those of Prof. B. G. Peters on "Parasitology at Imperial College"; Prof. J. G. Ball on "Metallurgy—Art, Technology, Science"; Prof. W. K. Hayman on "Pure Mathematics at a Technological University"; Prof. Abdus Salam, "Elementary Particles"; Prof. P. V. Danckwerts, "Chemical Engineering in a University"; Prof. D. H. R. Barton, "Some Modern Trends in Organic Chemistry"; Prof. C. C. Butler, "Ultra-High Energy Nuclear Physics"; Prof. F. D. Richardson, "The Changing World of the Extraction Metallurgist"; and Prof. J. M. Kay on "The Nuclear Power Programme and its Impact on Engineering Education" (pp. ii+134+24 plates. London: Imperial College of Science and Technology, 1959).

University News: Birmingham

THE University of Birmingham and the University College of Rhodesia and Nyasaland have agreed that when a Medical School is established in the University College at Salisbury, Southern Rhodesia, this School will initially be an affiliated institution of the University of Birmingham. This will mean that, for the period of affiliation, students in the Medical School of the University College will be prepared for degrees in medicine of the University of Birmingham.

The following lecturers have been appointed: J. C. Maltbaek (chemistry); Dr. D. S. Urch (chemistry); G. C.-Y. Hu (highway and traffic engineering); Dr. C. G. Wall (chemical engineering); G. H. J. Bennett (industrial metallurgy); R. S. J. Good (mechanical engineering).

Cambridge

DR. KENNETH MCQUILLEN, University lecturer in biochemistry, has been appointed College lecturer in biochemistry in Churchill College as from October 1961.

Durham

THE University of Durham has received a grant from the University Grants Committee to enable it to place orders for nearly £40,000 worth of additional equipment for the University Computing Laboratory at 1 Kensington Terrace, Newcastle upon Tyne 2. At present, the Laboratory is equipped with a Ferranti Pegasus electronic digital computer, and this installation is to be enlarged by the addition of magnetic tape equipment and of a fast output punch.

London

DR. R. G. LIPSEY has been appointed to the University readership in economics tenable at the London School of Economics and Political Science. The title of reader in economics in the University of London has been conferred on Dr. K. J. Lancaster in respect of his post at the London School of Economics and Political Science.

Announcements

THE Messel Medal, the senior award of the Society of Chemical Industry, which is awarded every two years in gold, will be presented to Viscount Chandos in Bristol on July 6 during the annual meeting of the Society. The Medal is awarded for meritorious distinction in science, literature, industry or public affairs.

PROF. E. J. WAYNE, regius professor of practice of medicine in the University of Glasgow, has been appointed chairman of the Clinical Research Board, in succession to Sir Geoffrey Jefferson.

DR. DAVID K. DETWEILER, associate professor of veterinary pharmacology in the University of Pennsylvania, has been awarded a grant of 1,100,000 dollars over a period of ten years from the National Heart Institute of the National Institutes of Health, for research and training in comparative cardiovascular studies.

THE International Society of Tropical Ecology, with its headquarters at Allahabad, India, was inaugurated by His Excellency the Governor of Bombay on January 5. The following officers were elected for 1960–61: *President*, Dr. J. C. Sen Gupta (Calcutta); *Vice-presidents*, Dr. F. R. Fosberg (Washington), Dr. C. G. G. J. Van Steenis (Holland); *Treasurer*, Dr. R. Misra; *General Secretary*, Dr. G. S. Puri.

AN international symposium on "Inhaled Particles and Vapours", organized by the British Occupational Hygiene Society, will be held in Oxford during March 29–April 1. Further information can be obtained from Dr. J. S. McLintock, Medical Service, National Coal Board, Hobart House, London, S.W.1.

A SYMPOSIUM on "Inorganic Chemistry" will be held in the Wallberg Building, Department of Chemistry, University of Toronto, on April 8. Further information can be obtained from Dr. A. D. Allen, Department of Chemistry, University of Toronto, Toronto 5.

A SYMPOSIUM on "Continuous Culture of Micro-organisms" will be held during March 31–April 1 at University College, London, under the auspices of the Microbiology Group of the Society of Chemical Industry. Further information can be obtained from the Assistant Secretary of the Society, at 14 Belgrave Square, London, S.W.1.

IN the review of the book, "Mathematical Methods of Operations Research", by T. L. Saaty, published in *Nature* of December 5, 1959, p. 1763, it was stated that a printing error occurred on p. 340. Mr. R. Syski, of Hivac, Ltd., South Ruislip, Middlesex, has pointed out that this is not the case, and Prof. L. S. Goddard agrees, adding that the formula is repeated incorrectly on p. 346, where the exponent of μ/λ in the second term is given as $i + n - 1$, whereas the correct exponent is $i - n + 1$.

THE NATIONAL MUSEUM OF WALES

DEPARTMENT OF INDUSTRY

A DEPARTMENT of Industry was founded in the National Museum of Wales, Cardiff, in 1958, and its gallery was opened to the public last December.

The purpose of the Department is to illustrate not only the history and development of the older Welsh industries that were founded upon Welsh raw materials or upon techniques evolved or developed in Wales but also the application of science and research to the industries more recently introduced. In presenting an objective record of technical progress an attempt will also be made to show something of its impact upon human life.

The exhibits are displayed in fixed cases of shop-window type and in standard museum cases. For the present, there is a preponderance of those dealing with the iron and steel industry in Wales; in some exhibits it is looked at from the historical point of view alone, whereas in others the contrast between old and new methods is brought out by combining models of obsolete plant and photographs of modern processes.

The first of the iron-making exhibits contains a model of an anthracite blast-furnace used in the Gwendraeth Valley about 1850, and one of a blast furnace with a stove for heating the air before its injection into the furnace. The latter was associated with the successful attempts of John Budd in adding pre-heating chambers to some anthracite-burning furnaces at Ystalyfera in the Swansea Valley in 1844. Like other contemporary blast furnaces, those at Ystalyfera were constructed of massive masonry enclosing and supporting the fire-brick chambers in which the reactions took place. A further typical example of the construction adopted in South Wales before 1850 is depicted in a model of a blast furnace at the Pen-y-Darren Ironworks. Included in the model is a small pipe stove, consisting of nine semi-circular cast-iron pipes placed in parallel in a chamber heated by a coal fire, by which some of the blast was heated. Coupled with this model is one of a blowing engine used at the works for supplying air to blast furnaces. The type of blast furnace erected at Cardiff between 1888 and 1895 is represented by a half-section model. These furnaces were hand charged until 1927.

Obsolete steel-making plant is illustrated by a model of an open-hearth steel furnace of a type used in South Wales from 1890 until 1900, and included in the same exhibit is a sectional model of a Siemens' gas-producer, formerly the property of Sir C. W. Siemens, representing the modified type of gas-producer which he devised for use with the open hearth process perfected by him in 1867. In an exhibit on steel-making by the Bessemer process, there is a sectional model of a Bessemer converter of 1856.

In 1898 the works engineer at Dowlais Works designed and patented an ingot-stripping machine. Previously the cast-iron moulds had been stripped from steel ingots by hydraulic and steam cranes working alongside the sunken teeming pits. The new stripping machine, hydraulically driven, made it possible for ingots to be cast on bogies and then taken to the soaking pits some distance from the steel-making plant. A model of this machine is on view, and there is also a model of the original engine used at the same works for driving the roughing rolls of the rail-mill.

The hand processes of making tinplates in works dismantled during recent years are illustrated in an exhibit containing tools, photographs and samples of plates in the various stages of rolling from the original tin bar to the finished pack of eights.

At one end of the gallery there is a small transport section showing models of nineteenth-century sailing ships, illustrating the transition from sail to steam, and of early twentieth century cargo steamers under Welsh ownership. There is a model of the first Garratt articulated locomotive built for use in Great Britain, which operated at the Hafod Copper Works, Swansea, and one of a Taff Vale Railway engine and tender of 1867. A working model of a high-pressure steam engine designed by Richard Trevithick is also included; it is of a type installed at iron-works at Pen-y-Darren, Hirwaun and Tredegar at the beginning of the nineteenth century. A small panel of photographs alongside this model illustrates parts of the remains of the tram-road from Merthyr to Abercynon along which the Trevithick locomotive travelled in 1804.

One of the fixed cases contains a model of a rotary cement kiln. This will be supplemented by an exhibit adjacent to it which will include specimens of the raw materials used in the making of cement, of the product at various stages of manufacture, and of photographs of quarries and parts of the plant involved. An exhibit of Welsh exposed-aggregate slabs is also planned. A collection of tools, photographs and drawings illustrates the preparation of roofing slates in North Wales, and there is an exhibit devoted to the history and development of the miner's safety lamp.

The rayon industry is represented in an exhibit illustrating the manufacture of rayon staple and box spinning textile yarn at a factory in North Wales. An exhibit dealing with the nylon industry is being planned, and it is hoped to have another featuring the nickel industry.

Some original machines—a beam engine for pumping water from pits, a water-balance pit-head gear used to raise coal, and a set of rolls and a shear-table from an old-type tinplate works—are installed in the grounds of the Museum.

The gallery as it now stands indicates the field which the Department of Industry is intended to cover. It will be added to as rapidly as circumstances permit; but full use of models and original objects now in reserve cannot be made, nor can the full illustration of the special industries of Wales be achieved, within the confines of the present gallery. Much of this work will have to await the hoped-for addition to the Museum of a west wing.

WATER RELATIONSHIPS OF PLANTS IN ARID AND SEMI-ARID CONDITIONS

THE part played by Unesco in stimulating interest in the problems of the drier regions of the surface of the Earth is widely recognized. Its excellent series of symposia, covering hydrology, utilization of saline water, plant ecology, human and animal ecology, wind and solar energy, and micrometeorology, has done much to crystallize our current concepts of water as an ecological factor and particularly to emphasize the physical background in which it operates. These discussions were extended to the level of the individual plant at a symposium organized in Madrid during September 1959.

Considered as a whole, the two published volumes of the Canberra symposium on micrometeorology and the two volumes of the Madrid symposium, which have yet to appear, provide not only one of the best modern treatments of plant–water relations but also illustrate clearly the way in which plant ecology is casting its traditional shackles and emerging as an active experimental discipline. The Madrid symposium, although leavened with a few soil physicists and plant physiologists, was numerically dominated by plant ecologists. Among these were included representatives of the three modern categories: (i) the 'chroniclers', whose penchant for description and cartography readily translates suggestions into irrefutable dogma and leads to the creation of beautiful but often meaningless maps—multivariate analysis apparently does not yet qualify for an expense account; (ii) the 'partialists', whose methods have not advanced beyond the era of Livingston and Maximov, and whose thought is restricted to the confines of the plant; and (iii) the 'totalists', whose concern is with the entire soil–plant–atmosphere system. It is no accident that the modern approach to experimental ecology represented by the third category should have developed within the applied science of agriculture but simply reflects the stimulus arising from close association between people investigating detailed aspects of different parts of the same system. One of the finest examples of an ecological investigation is that reported at Madrid by R. O. Slatyer on the water relations of two natural communities in Australia. Here, soil physicists, plant physiologists and micrometeorologists are working together and each has a full appreciation of the complete system.

It is also no accident that this approach should emerge first with the study of water because the responses of the plant to water are less complex than to other ecological factors such as light or temperature. Knowledge of the control of the water supply has now advanced to the stage of complete quantitative description, although the values of some parameters are far from exact. Those which are least perfectly known include moisture-release curves, surface roughness, stomatal conductance and the variations in extent of the root-zone with time, species and environment. Several contributors discussed these. The location and sizes of the resistances in the plant to liquid flow are uncertain, although it is now clear that plants may experience an adverse deficit of water in the leaves with the soil water at 'field capacity' when the potential transpiration exceeds ~ 4–5 mm./day. In order to evaluate this and other problems intelligently and to remove general confusion, the status of water in all parts of the soil–plant–atmosphere system must be expressed in equivalent terms. The logical terminology is that of thermodynamics, and all members of the conference unanimously agreed to use energy terms and units henceforth. The *water potential* (difference in free energy between the water contained in the soil or plant system and pure free water at atmospheric pressure) should be used for the quantity normally associated with total soil-moisture stress and diffusion pressure deficit or suction pressure. It is determined by three components: (1) the *osmotic potential*, which takes account of all dissolved materials in the soil solution or plant sap and is usually called the osmotic pressure; (2) the *pressure potential*, which is associated with the total pressure difference in the system, that is to say, with the difference in pressure between the water in a tensiometer and that in the adjacent soil or with the turgor pressure of plant cells; and (3) the *matric potential*, which depends on the solid matrix and the forces of retention of water in the matrix and which has been associated with terms previously called soil suction or moisture tension but which is usually ignored in plant systems.

Knowledge of the response of plants to water supply is in a less happy position than is our appreciation of transpiration. The differing responses between species, and within the one species with phase of growth, to the apparent soil–water deficit arise mainly from differences in the rate of extension of the root system, from measuring the deficit at one or two depths only, and from the difficulties associated with obtaining an integrated value of the water deficit in the soil actually occupied by roots. With increasing values of the water deficit in soil fully permeated by roots at any point of time, growth appears to decrease over the range of water potential from $\sim -0{\cdot}01$ ('field capacity') to $\sim -1{\cdot}5$ joules/gm. ('wilting point'), but the form of the relationship is modified by the level of potential transpiration and other environmental aspects. R. M. Hagan, N. S. Petinov and S. A. Taylor discussed irrigation within this context.

Survival under dry conditions—the problem of drought resistance—is still little understood. The mechanisms involved, discussed by H. R. Oppenheimer, A. M. Migahid and other contributors, are complex and numerous. Some of these, such as extensive root systems, leaf fall and stomatal closure associated with low cuticular conductance, prolong the period during which the water supply of tissue capable of growth can be maintained. The reaction of cells and tissues to drying, analysed by O. Stocker, also differ, and the behaviour of the whole plant is related to the resultant of the complex interactions which occur between varying proportions of cells of differing responses. One of the most exciting developments in this field is the hardening of plants prior to sowing which has been developed by Soviet scientists.

Increases in yield from 10 to 40 per cent are claimed. These and other contributions from the U.S.S.R. to the understanding of the reactions of plants to water and to heat were discussed by N. S. Petinov and P. A. Genkel.

The discussions will be published in two parts: (1) Reviews of research prepared as background material on "The Income and Loss of Water" (F. L. Milthorpe), "Soil Water Relations" (W. R. Gardner), "Physiological and Morphological Changes in Plants due to Water Deficiency" (O. Stocker), "Adaptation to Drought—Xerophytism" (H. R. Oppenheimer), "Methods of Research on Water Relations" (F. Eckardt), "The Management of Native Vegetation" (R. M. Moore), "Principles of Dry Land Crop Management" (S. A. Taylor), "Significance of Fallow as a Management Technique" (W. J. Staple), "Principles of Irrigated Cropping" (R. M. Hagan and Y. Vaadia), "Survey of Research on the Water Regimes of Plants" (P. A. Genkel and K. A. Bodanova), "The Water Regime of Plants on Saline Soils" (B. P. Strogonov); (2) Proceedings of the symposium, which include about forty papers contributed on detailed aspects within the above fields by workers from seventeen different countries.

F. L. Milthorpe

MINING GEOPHYSICS

THE second Nordic Meeting for Mining Geophysics was held at Haandværkerforeningen, Copenhagen, during December 7–8. More than fifty geophysicists and technicians, including five from Finland, eleven from Norway, and eighteen from Sweden, attended. The sessions were presided over by Haakon Brækken from Trondheim, Norway, chairman of the planning committee.

The technical programme included twelve papers, two of which were concerned with Greenland. B. Sjögren (Sweden) reported on a seismic refraction and reflexion investigation on the Malmberget glaciers. The area surveyed lies about 20 km. south-east of Mestersvig, Kong Oscars Fjord, east Greenland, and the purpose of the survey was to determine the thickness of the ice–moraine layer of the glaciers. The bed-rock is mainly sandstone from the Carboniferous period which is superseded by tertiary granite. The bed-rock velocities measured varied from 4,800 to 5,400 m. per sec. while the longitudinal wave velocity in the ice–moraine layer was between 3,700 and 3,800 m./sec.; the transverse wave velocity in this layer was of the order of 1,600–1,700 m./sec. The measured thickness of the ice–moraine cover varied from 120 to 450 m. The bed-rock under the glaciers was discovered to be U-dale shaped with relatively shallow slopes while the rock walls on the sides of the glaciers climbed very steeply to about 700 m. above the glacier surface. The second paper dealing with Greenland, "A Study of the Disturbances due to Salt-water on Minor Loop-frame Indications", was presented by J. Bondam (Denmark). The survey is part of experimental measurements carried out near Narssaq, south-west Greenland.

The important problem of the elucidation of structure was illustrated by G. F. Sakshaug (Norway). Electromagnetic, magnetic, and self-potential surveys have been employed in the exploration of copper mineralization at Bidjovagge, Finmark (Northern Norway). Electromagnetic surveys have been conducted as a general reconnaissance method as well as towards the examination of detail. Electromagnetic anomalies are largely confined to beds of graphite shale occurring in the area. Magnetic surveys define magnetite-bearing greenstones. Self-potential surveys give essentially a map of the graphite shale under the moraine cover. All three methods have been essential to the interpretation of the structural relations in the area. Irregularities in the anomalies reflect tectonic disturbances, and ore-mineralizations—as diamond drilling has confirmed—are related to areas of intense disturbance. Another example of structure mapping was given by V. Münther (Denmark) in a presentation of a magnetic survey of Bornholm and its application to the geology, especially the tectonics. The island has been covered by magnetic stations in squares, the side-length being 500 metres. Moreover, a considerable number of traverses has been established, stations distances being from 10 to 20 m. apart. It has in this way been possible to correct the previous boundary lines between granite and sandstone. Known faults have been traced in more detail and older faults have been shown. In the granitic area the magnetic contours indicate the granite variety. Interpretation of the anomaly map indicated partly stronger magnetic layers at a greater depth, partly a change in the magnetic field with time. The practical aspect of the survey is the economic interest in sandstone.

A case-history was demonstrated by J. Espersen (Sweden). Magnetic and gravimetric measurements have pointed out the existence of two important and several minor ore bodies at Svappavaary near Kiruna, north Sweden. The applicability of magnetometers and gravimeters in ore prospecting is underlined by the confirming drilling results. J. Nuutilainen (Finland) described gravimetric application in ore prospecting. Levelling is carried out by means of a mercury pressure-gauge and a water tube which at the same time is the measuring tape. H. Paarma (Finland) considered magnetic gradient measurements with regard to ore bodies and structures. A report of interest concerning the conductivity of magnetite, sulphide, and different rocks sampled in Swedish mines was given by Sture Werner (Sweden). Measurements were undertaken in fifteen sulphide-mines and twenty iron-mines. The resistivity, which is the reciprocal of the conductivity, was found to amount to 300,000–3,000,000 ohm-cm. for the circumjacent rocks, while the value for magnetite and sulphide was usually found to be of the order 0·01 ohm-cm. The resistivity increases rapidly as the percentage of magnetite decreases; for example, an ore sample with 50 per cent iron has a resistivity of about 10,000 ohm-cm. For the sulphide samples the resistivity increases slowly as the percentage of sulphide decreases. Only at a very low sulphide content do the resistivity values rise.

A new instrument for measuring the curvature in bore-holes was presented by A. Holm (Sweden), and A. Breen (Norway) discussed the employment of phase-sensitive voltmeters in measuring electromagnetic fields. A point of practical importance was

reported by B. Karlemo (Sweden) in a description of methods for production of geophysical maps.

At a joint evening meeting with the Danish Geophysical Society, D. Malmqvist (Sweden) gave a review of electrochemical ore-prospecting methods and emphasized the utility of this prospecting method. The 'polarization provoquée' method proposed by Schlumberger about forty years ago seems to be very suitable, especially in cases dealing with disseminated ores, and Mr. Malmqvist demonstrated some survey examples from northern Sweden.

The chairman, H. Brækken (Norway), introduced at the business meeting the very important problem of education of mining geophysicists. The meeting appointed a committee (Brækken, Werner and Tengstrøm (Sweden)) to consider the different points and to report at the next meeting.

With regard to the planning committee for the third meeting, which is contemplated within two years, the meeting appointed B. Tornqvist as chairman (Sweden), and H. Brækken, M. Puranen (Finland), and S. Saxov (Denmark) as members.

In all, the second Nordic Meeting for Mining Geophysics proved to be most successful, the attendance being about 100 per cent higher than at the first meeting at Boliden, Sweden, during November 7–9, 1956; the discussions were many and the opinion of the meeting strongly supported the arrangements for future meetings and co-operation in common problems. SVEND SAXOV

THE MEDICAL RESEARCH COUNCIL

REPORT FOR THE YEAR 1957–58

THE recent report of the Medical Research Council for the year 1957–58, prepared by the Committee of Privy Council for Medical Research, adopts once more the form now used for several years (Cmnd. 792. Pp. viii+288. (London: H.M. Stationery Office, 1959.) 13*s*. net). Instead, that is to say, of giving detailed accounts of all the researches in progress, it gives articles based on certain aspects of the Council's very varied programme, the intention being to deal, once every few years, with all the important fields of research undertaken by the Council's staff. These articles are also published separately under the title "Current Medical Research" (Pp. iii+46. (London: H.M. Stationery Office, 1959.) 3*s*. 6*d*. net).

In this publication and also in the report itself we are given a description of the Council's Laboratory in Gambia, West Africa. As part of its contribution to research in tropical medicine, the Council maintains three laboratories, in Uganda, Jamaica and Gambia, respectively, and that in Gambia here described is the oldest and largest. Unlike the others, it is self-contained and is not attached to any local institution. Situated at Fajara, it was originally part of the Council's Human Nutrition Research Unit in London, but its scope has now been widened to include research, not necessarily on tropical medicine, but in other fields as well. Many research workers use its increased facilities—cardiologists, chest and other specialists, pædiatricians, public health workers and others. The centre is visited by many research workers from Britain and has lately been used by a team representing the Council's Research Group on trachoma, a cause of blindness which, because of the menace it is, is engaging the attention of research workers all over the world. This centre is also developing research on the hyperendemic malaria of the Gambia region, a region which offers special facilities for work of this kind, and this is discussed in a separate article in the report. Much of it is done at Keneba, and to facilitate communication between Keneba and the laboratories at Fajara a radio-telephone has been set up, and the Wellcome Trust has generously provided a cabin-cruiser, *The Lady Dale*, which can also be used as a floating laboratory.

As well as the article just mentioned on the hyperendemic malaria of the Gambia area, the report includes articles on the remarkable work being done by members of the Council's staff on intracellular organelles and on the enzymes contained in them. Other articles discuss the work being carried out on the viruses of trachoma and inclusion blenorrhœa, the types of the bacillus that causes Sonne dysentery, the cancer-producing viruses and their immunology, the ultra-microscopic analysis of body fluids, the performance of coal miners in hot atmospheres, the fertilization of the mammalian egg and the rehabilitation of schizophrenic people. Another article of interest discusses research in general medical practice, a line of work which is much to be encouraged. An innovation in the report is the introduction of two photographs, one of which shows *The Lady Dale*, and two line illustrations.

For the rest the report contains its usual features. Brief summaries of the work of each of the Council's research units are given, together with the names and research addresses of the Council's many workers. A long bibliography lists the varied work that these workers have published. G. LAPAGE

COMPARISON OF RAIN-GAUGES

THE measurement of precipitation is a difficult problem because of the great variations in the nature of the precipitation and of the effects of wind eddies around the gauge. The radii of liquid drops and their rates of fall range from 0·2 mm. at 0·7 m./sec. of drizzle to 5 mm. at 8 m./sec. of the heaviest thunderstorm rain, while the speed of the wind around the gauge can vary over a somewhat greater range. Solid precipitation is even more difficult to measure. It ranges from fine snow to heavy fast-falling hail-stones and may at low temperatures completely choke the gauge if, as is usual, no heating

is provided for melting. In addition to these inherent difficulties, rain-gauges must be cheap and simple to operate because, on account of the great variations in rainfall from place to place, it is necessary to have many of them widely distributed. Different types of gauge have been evolved in different countries in the endeavour to meet these difficult requirements.

The ordinary British type is fixed in the ground and has a catching cylinder with a diameter of 5 in., and which is set with the rim 1 ft. from the ground. Some Continental countries fix rain-gauges to posts so that the rim is 1 m. to 1·5 m. above the ground. The shapes of the interior of the cylinders differ considerably, while in some countries the catching cylinder is contained in a much wider coaxial cone called a shield intended to minimize the effects of wind eddies.

One of the basic principles of meteorological measurement is that observations, though their absolute accuracy is to some small extent inevitably uncertain, shall be comparable. With this aim in mind, the Royal Meteorological Institute of Belgium has recently completed a five years comparison of Belgian, British, German and French rain-gauges at the Uccle Observatory near Brussels and at Yangambi in the Congo. The work is described by Dr. L. Poncelet in a recent publication by the Institut Royal Météorologique de Belgique (Publications Série A. No. 10 : Sur le Comportement des Pluviomètres. Pp. 58. Uccle-Bruxelles : Institut Royal Météorologique de Belgique, 1959). The results are analysed according to season, intensity and continuity of fall, occurrence of sunshine after rain (because of evaporation of some of the catch), and wind speed and direction. The amounts collected in individual months vary greatly from gauge to gauge and a long period of averaging is needed to produce a consistent pattern of differences. Over the whole period the differences were less than 1 per cent, with the German and French high-level gauges giving lower readings than the low-level Belgian and British gauges. Dr. Poncelet considers that different types of gauge can be compared to an accuracy of 1 per cent, and that the relation of the annual totals to the precipitation which would actually reach the ground where the gauge stands during a year is of the same order of accuracy. The accuracy is appreciably less for snow at 2–3 per cent.

Dr. Poncelet, in his capacity as chairman of the World Meteorological Organization Working Group on the International Comparison of Precipitation Gauges, has also written an article in the October 1959 edition of the World Meteorological Organization's *Bulletin* on the use of a specially designed international reference precipitation gauge. This gauge is fixed in the ground and has its rim at a height of 1 m. and is contained in a conical coaxial shield the width of which at rim-level is 1 m. Arrangements are in hand for comparisons to be made in most countries between the readings of this reference gauge and the national gauges. The latter may, it is believed, differ from one to another by 5–15 per cent. Most of the reference gauges will be made by the same (British) manufacturer. In any event, every effort is being made to ensure uniformity down to the degree of polish of the copper surface and the wetting properties of the polythene receiving bottle and measuring glass.

It is hoped that this comparison, which will take several years, will provide means of reducing rainfall observations for comparison with an accuracy of 1–2 per cent, which will permit computations of water balance to be made and rainfall maps drawn without discontinuities at national frontiers.

G. A. Bull

MEASUREMENT OF VISUAL RANGE

By Dr. P. CROSBY

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TWO photoelectric instruments have been constructed at the Weapons Research Establishment, Salisbury, South Australia, for measuring the scattering coefficient, b, of the atmosphere. Because the associated 'visual range' V is more commonly understood, it is our practice to quote the results as 'visual range' values, where $V = 3{\cdot}912/b$ (see ref. 1).

Polar nephelometer. The first instrument is a version of Waldram's polar nephelometer[2]. The light source is an ionic flash tube. The light beam is defined by a baffle-box with square apertures. A second baffle-box with a photomultiplier tube forms the photometer. The apertures are square and the field of view of the photometer passes through the light beam. The photometer is rotated about a fixed point of the light beam to receive light scattered at various angles, from 15° to 165°. The volume of illuminated atmosphere that lies in the field of view of the photometer being known from the dimensions of the apparatus, a polar diagram of scattering of the light flux scattered by unit volume of atmosphere is obtained and extrapolated over the regions 0°–15° and 165°–180°.

The polar diagrams show a strongly forward scattering, indicating that most of the scattering is from large particles.

A measurement of the intensity of the source is made by directing the photometer at the source with a very dense neutral filter in the light-path. Integration of the polar curve produces a value of b or V.

The filter is required to prevent overloading the photomultiplier. Its transmission factor is calibrated against the reflexion of light from two magnesium oxide surfaces.

Integrating nephelometer. The second instrument is a version of the Beuttell and Brewer integrating nephelometer[3]. The light-source behind a piece of opal glass is an ionic flash tube. The photometer consists of a photomultiplier tube and baffle box with circular apertures. It is directed across the opal glass towards a black box in order to receive light scattered at angles between 10° and 170°. A correction of 6–7 per cent is required to allow for light scattered between 0°–10° and 170°–180°, according to the polar nephelometer results. The

amount of light flux reaching the photomultiplier is directly proportional to the scattering coefficient.

A reference measurement of the intensity of the source measured along the normal to the opal glass is required to obtain absolute values of b. The intensity is measured by placing a diffuse reflector above the opal glass, on the axis of the photometer, to direct light through a dense filter on to the photomultiplier.

The photomultiplier output of either instrument is measured as the voltage across a 10^5-ohm anode load using a cathode-ray oscillograph with a long-persistence tube.

Meteorologists should be particularly interested in the integrating nephelometer since it is a direct-reading instrument. First, the voltage applied to the photomultiplier is adjusted to produce a standard cathode-ray oscillograph deflexion when measuring the intensity of the light source. Then, when the photometer is receiving scattered light, the 'visual range' is read directly by noting the cathode-ray oscillograph deflexion on the special scale placed over the screen. The instrument is 7 ft. × 18 in. × 18 in. and costs less than £200, excluding the cost of the two power supplies and the cathode-ray oscillograph.

Observations are made inside a well-ventilated room. Complete darkness is not required. Experience has proved that the results obtained indoors are identical with those outdoors within the 10 per cent accuracy claimed for the system, on dull days and at night-time. Checks during sunlight are not possible, as there is too much background stray light.

The visual range fluctuates widely during any one day. Generally, visibility is between 25 and 45 miles. Lower readings are obtained on occasions. The least was 0·5 miles during a mild sandstorm. The highest was 53 miles after heavy rain had fallen. The equipment is to be transferred to Woomera to study conditions there.

The 'visual range' is, according to meteorological definition, the distance at which a dark object can be distinguished and recognized against the horizon sky. It should be noted that seldom has a meteorological office any special markers and that generally a glance outside is the only basis of the 'visibility' figure published by the office. Sometimes an inspection of various distant landmarks is made, but estimates by different observers vary widely due in part to the different luminal contrast of their eyes. In any event, the ability to see a distant object depends on the prevailing background brightness in that direction, and the presence of cloud would affect the result.

The nephelometers, on the other hand, make a direct measurement of the basic property of the atmospheric scattering which is the primary cause of loss of contrast or brightness of an object as it recedes from the observer.

Special features of the new nephelometers compared with the visual instruments are :

(*a*) Use of an ionic flash tube. This produces a high-intensity light beam which need not be concentrated further by mirror or lens systems. The latter would have surfaces on which dust could collect and form stray light. The heat energy within the light beam from an ionic flash tube is not sufficient to dry the sample of atmosphere. Continuous sources, used in visual nephelometers, produce a great deal of heat.

(*b*) Use of photo-multiplier tube. This eliminates the fatigue of visual measurement of the low intensities of scattered light.

(*c*) Automatic recording of visibility. The visibility is read directly off the cathode-ray oscillograph screen of the integrating nephelometer after the calibration has been carried out. The light source can be operated automatically and a continuous photographic record of the screen made. Alternatively, the cathode-ray oscillograph can be replaced by a pen-recording system. In either case, a chart similar to a temperature-time chart is produced and would be valuable for forecasting visibility.

This communication is submitted by permission of the Chief Scientist, Australian Defence Scientific Service, Department of Supply, Melbourne.

[1] Middleton, W. E. K., "Vision through the Atmosphere", 105 (Toronto Press, 1952).
[2] Waldram, J. M., G.E. Co. Ltd. Rpt. 8064, and Middleton (above), p. 201.
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IONIC EXCHANGE AND THE STRUCTURE AND FUNCTION, INCLUDING MOTILITY, OF CYTOPLASMIC MEMBRANES

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CONSIDERABLE study has thus far yielded no adequate unified explanation of the structure and function of superficial cytoplasmic membranes. Various metabolic, or carrier, hypotheses have not been substantiated when tested. Other chemical explanations, such as the lipoid – sieve hypothesis which views membranes as static structures, are limited to certain aspects of permeability. Our present increased knowledge of ionic exchange and the realization that macromolecular constituents of the cytoplasm are capable of functioning as ionic exchangers present the possibility of an explanation of membrane structure and function based on certain principles of ionic exchange.

A key problem is the formulation of an explanation of the conversion of the chemical energy of metabolism to cellular motions ; one possible explanation is a reversible hydration – dehydration of the membrane. This sort of change can be an effect of certain types of ionic exchanges and may be influenced by a

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variety of compounds. As outlined below, this hypothesis, that ion-exchange phenomena may produce reversible hydration, permits a unified explanation of the electrical behaviour of cells, of differential permeability and of membrane motility.

Because of the relatively recent applications of ionic exchange principles to biology, a few general points about them should be reviewed. There are two types of cationic exchangers: strongly and weakly acidic; and two types of anionic ones: strongly and weakly basic. Particles of ionic exchange materials consist of insoluble frameworks, permeable to water and with firmly bound acid or basic functional groups. Ion exchange depends on charge density: trivalent ions displace divalent ones, and so on. Concentration of ions is also a factor. Cross-linkage is the term for the rigidity of bonding within exchange particles. The specificity of exchange depends on cross-linkage and properties of the compounds making up the exchanging particles. Rates of exchange decrease with increases in cross-linkages. Exchange-rates can be decreased by the binding of large ions on particle surfaces[1].

Biological materials may act as ionic exchangers[2]. Proteins, acid mucopolysaccharides and cerebrosides are weakly ionized, whereas the sulphated mucopolysaccharides, nucleic acids and phospholipids represent strongly ionized exchangers. Proteins exchange or bind ions such as hydrogen, calcium and the polyvalently ionized macromolecules. Fibrous proteins, long believed to form part of membrane structure, may also exchange hydrogen ions for sodium and potassium. However, the exchangers appear to work better in an insoluble form. Hence, it may be of interest that these biological exchange materials can exist in the cytoplasm as particles.

There seems to be good circumstantial evidence that the membranes are composed of some sort of cytoplasmic particle. It has been possible to demonstrate both the motility of cytoplasmic membranes and pinocytosis[3]. Certain cells have been shown to take up foreign proteins by phagocytosis, pinocytosis or both[4]. These processes are something of a puzzle, implying as they do not only a transient rupture of the cytoplasmic membrane but also a rather sudden increase in the amount of membrane material as well. If membrane material is potentially particulate and if the particles are drawn from the cytoplasm as needed, these puzzles can be solved. This implies that there are cytoplasmic particles that have some properties in common with membranes. Outstanding properties of membranes are permeability to 'fat-soluble' materials and electrical phenomena; the former implies a high content of lipid, whereas the latter implies ionic exchange because of an absence of metallic conductors. Microsomes have a high content of lipid and of nucleoprotein, so they may take a role in membrane formation. However, electron-micrographs which show the superficial cytoplasmic membranes and the mitochondria do not reveal any structures that can be clearly identified as microsomes[5,6]. Perhaps microsomes are in some measure created by the stress of high-speed centrifugation, and, if so, these particles and membranes may have a common precursor. Because membranes act as a lipoid phase, it is plausible that these particles form the membrane by coalescence of their lipids.

Assuming the existence of these membrane particles, one may indicate the effect hydration would have on permeability to water-soluble substances. Hydration of the lipids of these particles, if it went far enough, would increase permeability. This permeability would not only involve materials such as the sugars, but also the water-soluble ions. Under suitable conditions a flow of ions would produce the electrical phenomena. Hence, if one understood how to hydrate or dehydrate the membranes, one could exert some measure of control over both permeability and bioelectrical phenomena. Parenthetically, one should note that these particles would be in constant motion relative to the fluids which bathe them and that this could produce a situation like that in an ion-exchange column.

Hydration and dehydration of the cytoplasmic membranes (either by direct introduction or withdrawal of water or else simply by altering its activity) imply changing composition. Introduction of fat-soluble organic compounds could affect the hydration of membrane lipids. Carboxylic acids would tend to bind hydrogen and calcium ions and thus cause some dehydration. Phospholipids, being relatively highly ionized, would tend to be hydrating agents, and they could also tend to draw other hydrated ions into the membrane. Carbonyl and hydroxyl groups would tend to be hydrating agents whether they occurred in glycolipids or steroids. Organic ions, being fat-soluble and attended by water of hydration, would tend to be hydrating agents. In the opposite direction the more nearly non-polar ethers, esters, hydrocarbons and noble gases would tend to promote dehydration.

Reversible hydration is central to the explanation of membrane motility. Under suitable conditions the membrane particles would function as weakly acid cation exchangers. Such exchangers may function as converters of the chemical energy of ion-exchange to mechanical work because they undergo large increases in volume when they exchange their hydrogen for some other ion, and large decreases in it following a second treatment with acid[1]. The basis for such effects would be the carboxylic acid groups of the membrane particles. An increase of permeability would, for obvious reasons, tend to increase metabolism. Since acid, for example, carbon dioxide, is an important end-product of metabolism, the hydrogen ions formed would tend to produce a decrease of permeability and particle shrinkage. This change would be followed by a decrease in metabolism or less acid. The hydrogen ions in membrane lipids could then be exchanged for other cations in the environment, and this would lead to swelling of particles, increased permeability and so on. These changes would give rise to bioelectric phenomena.

A hydrated membrane, because of permeability to ions, would have a low electrical resistance and might be thought of as being depolarized. As acid from metabolism displaced cations from it, the membrane would become less hydrated and offer greater electrical resistance. The binding of hydrogen ions by the membrane would leave an excess of anions in the cytoplasm which would thus be negatively charged. Depolarization might be achieved by introduction of fat-soluble ions such as acetyl choline[7] into the membrane. Depolarization-levels of such ions would depend on the relative proportions of the various 'hydrating' or other agents in the particles.

The explanation of differential permeability is ordinarily to be subdivided into two parts: selective accumulation (or the reverse) of ions and of un-

ionized substances. Differential permeability to ions may possibly be explained as an exchange or displacement process. The sort of mechanism involved (bearing concentrations in mind) may be illustrated by a displacement series of cations, which is arranged in order of decreasing ability to displace crystal violet from a weakly acid silicate exchanger, as follows: putrescine, cadaverine, pyridoxine, thiamine, histamine, hydrogen, barium, acetyl choline, calcium, magnesium, prostigmine, atropine, epinephrine, and esserine[3]. The arrangement follows the principles of ionic exchange and 'ion exclusion'.

The explanation of differential permeability to unionized materials requires the introduction of another idea from ionic exchange. This concept (absorption or ion-exclusion) is the absorption of slightly ionized or uncharged molecules by ion-exchanger particles. Some simple rules apply to this absorption. Aromatic acids are more strongly absorbed than aliphatic ones. Non-polar substituent groups enhance and polar ones diminish this absorption. Absorption processes have made it possible to separate isomeric six-carbon sugars from each other (borate exchangers) and, by means of exchangers with optically active functional groups, it has been possible to separate optical isomers[3]. Absorption, since it can help to account for the differential binding and exchange of optical isomers, aromatic compounds, polyhydroxy compounds and lipids *in vitro*, may account for the binding of lipids to a variety of multivalent macromolecular ions in the formation of cytoplasmic membranes. If, for example, it is possible to displace one hexose by another on a borate exchange column, it would be possible to displace one hexose by another in a membrane particle where the role of borate was taken by some other residue. This is a possible mechanism of differential permeability of cells to un-ionized water-soluble compounds and an alternative to the 'carrier molecule' hypothesis.

Proposing an alternative to the metabolically derived carrier hypothesis means considering metabolism to be only indirectly connected with membrane function. The action of cyanide on mammalian erythrocytes illustrates, experimentally, the limitation on the metabolic interpretation of cell function. The action of cyanide has been assumed[9] to be due to the inhibition, demonstrated *in vitro*[10], of cytochrome oxidase. However, cyanide has also been implicated as having an effect on cytoplasmic membranes; Brinley's *Amoebae* survived injection of a dose of cyanide that was lethal, extracellularly[11].

The effect of cyanide on active transport of cations and glucose utilization in erythrocytes during an 18-hr. incubation period was studied by Maziels[12]. While cyanide in concentrations up to 10 mM/l. did not inhibit active transport, the potassium – sodium ratio was smaller and the amount of inorganic phosphate liberated was greater in cells treated with cyanide than in controls at comparable pH levels. Also, the amount of glucose utilized was greater in the cells treated. The implication of an increase in general permeability was noted in the results of this work on glucose utilization, phosphate liberation and cation ratios. Since cytochrome oxidase is of no consequence in red blood cells, the probable effect of cyanide would be to bind hydrogen ions. In terms of my hypothesis, this would lead to increased permeability.

The indication for further study along this line is clear. As the ion-exchange hypothesis provides a better explanation than does the cytochrome–oxidase-inhibition theory for the action of cyanide on mammalian erythrocytes, so through 'ion exclusion' or absorption a better understanding of structure – action relationships in drugs may be possible. Indeed, in the general application of ion-exchange phenomena to aid our understanding of biology, we may be seeing the start of a rational approach to such fields as immunology, oncology and neuropharmacology.

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SCRAPIE: A TRANSMISSIBLE HEREDITARY DISEASE OF SHEEP

By H. B. PARRY

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SCRAPIE is a chronic wasting syndrome affecting sheep in middle age, well known in Western Europe for at least two hundred years, almost always fatal and associated with degenerative changes in several organ systems. Its cause remains unknown; it can be transmitted experimentally by inoculation and some have considered the evidence available to favour a virus infection with a long incubation period[1], others a hereditary disease[2]. The literature has been reviewed recently[3].

The epidemiology of scrapie has been studied continuously over a 7-yr. period in 70 flocks in Britain of 10 breeds with some 10,000 breeding ewes; many of the flocks are fully recorded and are closed populations except for new sires[2]. Some clinical and pathological criteria of the disease have been described[2]. Only unequivocal cases of classical-type scrapie are included in this study, which is based on 800 cases, of which the great majority were in females, only 40 being in rams. 149 cases have been autopsied. Affected animals have not been isolated in quarantine, but their progeny and close relatives have been disposed of so far as has been practicable.

The results may be summarized as follows:

(1) *Age of Manifestation.* Data are available for 526 animals—492 females and 34 males. The age of manifestation varied from 1½ to 11 years in females and 1½–5½ years in males; the median age in both sexes was 3 years, the mean age being 3 years in males and 3½ years in females. 8·2 per cent of all cases were manifest by 2½ years of age, 71·9 per cent by 3½, 88·0 per cent by 4½, 93·4 per cent by 5½ and 97·5 per cent by 6½.

(2) *The 90 per cent Manifestation Level.* As 87·6 per cent of the females and 97·0 per cent of the males manifested the disease by 4½ years old this age has been adopted, for the purpose of classification, as an arbitrary end-point at which about 90 per cent of the potential cases of scrapie may be expected; the 10 per cent developing after 4½ years of age are termed late manifestation. An affected animal is one alive and well at 4½ years old and not manifesting scrapie thereafter while under observation; an unaffected animal not under observation until 4½ years old is termed unclassified. An affected animal is one manifesting the disease at any age.

(3) *Sex Incidence.* The disease would appear to affect both sexes in similar proportions, although the data for males are few.

(4) *General Epidemiology.* The flock incidence, that is, the number affected per 100 breeding females per annum, varies widely from flock to flock, even of the same breed, and from year to year. Many flocks may be considered as free, that is, no manifest cases for at least 5 years; in many the incidence is low (1–3 per cent) or sporadic (< 1 per cent), in some it is medium (4–10 per cent) and in a few flocks it is high (> 10 per cent), and may exceed 20 per cent.

A group of flocks, each flock breeding all its own females and selecting most of its sires from flocks within the group, may be considered to be a closed breeding population or flock-group. The flock incidence varies widely between different flock-groups; several have had no cases, others many.

In an affected flock-group, many flocks remain free of the disease, although they have used sires reared in medium incidence flocks during a period when manifest cases were present.

The introduction into a free flock of a sire which afterwards manifested the disease while in the flock has not been followed by any further manifestation of scrapie, in one instance for 2½ years and in another for 1½ years thereafter.

Where sheep of another flock-group free of the disease are run on the same farm and intermingled with affected sheep, no case of scrapie has appeared in the free flocks over 3–5 years.

In recorded flocks with a medium to high incidence initially, the incidence has dropped to the low range in all but three flocks. In two of these it has not been possible to dispose of close relatives; in the third a low incidence has suddenly risen to the high level, coinciding with the female progeny of certain matings reaching the age of high manifestation.

Many flocks with a low or sporadic incidence initially have had no case for 2–3 years or longer.

We have found no evidence to suggest spread of the disease from affected animals to others of the same generation by contact, coitus or contaminated environment.

(5) *Familial Incidence.* While in individual flocks of low to medium incidence affected animals appeared to occur capriciously in different and unrelated families, when these data were studied with knowledge of the performance of the whole flock-group, it became apparent that the incidence among individuals with a close relative affected, that is, a full or half brother or sister, father, mother, aunt, uncle or grandparent, was very much higher than in individuals with no close relatives affected.

The incidence in a female age-group, that is, in the classified progeny born into one flock in one year, usually sired by 1 or 2 rams, is more variable than the flock incidence, and may exceed 60 per cent.

The incidence among the progeny of one affected parent is higher than the corresponding flock and age-group incidence; in free or sporadic incidence flocks it is usually 0–5 per cent, in medium incidence flocks 25–30 per cent, and in high incidence flocks 50 per cent.

An unaffected female producing affected progeny by one sire will usually produce unaffected progeny by other sires at previous and subsequent matings, and the pedigree of her descendants will show occasional affected individuals.

A pedigree derived from an animal with one affected parent will often show a generation unaffected, but the disease usually appears again within 2–3 generations.

The incidence does not appear to be influenced by order of birth or foster rearing of the progeny. The progeny born to mothers manifesting scrapie at the time of their gestation and suckling are often but not always affected; whether the incidence is significantly higher than might be expected is not known.

(6) *Heritability.* The manifestation of scrapie among 232 sheep coded according to the performance of their parents is shown in Table 1; the incidence observed follows closely that expected of a hereditary disorder controlled by an autosomal recessive gene.

Table 2 summarizes data for sheep with two affected parents, and for those sired by an affected ram in a flock which has had no other case of scrapie for at least 30 years, and in which the dams may therefore be presumed to be mostly homozygous 'clear'.

The occurrence of scrapie among 34 sets of gestational, probably binovular, twins is consistent with the data shown in Tables 1 and 2. One set born of 2 affected parents were both affected: of those born of an affected and an unaffected 'carrier' parent, sometimes both are affected, sometimes one and sometimes neither; while of 18 sets born of unaffected 'clear' parents, all were normal.

Of 49 progeny of affected sires and unaffected dams, 13 (26·2 per cent) were affected; of 5 progeny of

Table 1. Manifestation of Scrapie observed among the Female Offspring of Parents with Varying Familial Connexions with the Disease, and that expected for a Simple Recessive Hereditary Disorder

Mating	Offspring		
	No. at risk*	No. affected observed	No. affected expected
Affected × affected	24	22	21·6
Affected × unaffected 'carrier'†	15	7	7·4
Unaffected 'carrier' × unaffected 'carrier'	6	0	~1·6
Unaffected 'carrier' × unaffected 'clear'‡	168	7	<8·2
Unaffected 'clear' × unaffected 'clear'	19	0	0

* Sum of affected plus unaffected animals under observation until the 90 per cent manifestation level at 4½ years old.

† Unaffected 'carrier' has one parent affected and/or more than one progeny affected.

‡ Unaffected 'clear' has no progeny or close relatives affected.

Table 2. Manifestation of Scrapie showing the Cumulative Totals of Affected Animals, observed and expected on the Basis of a Simple Autosomal Recessive Character, among the Offspring of (*a*) Two Affected Parents, and (*b*) One Affected and One Unaffected 'Clear' Parent

Age in years	½	1½	2½	3½	4½	5½	6½
(*a*) No. at risk*	93	63	42	31	30	29	28
Manifestation factor	0	0	0·08	0·72	0·88	0·93	0·97
No. affected Expected	0	0	3·4	22·3	26·4	26·1	27·1
Observed	0	0	5	23	25	27	27
(*b*) No. at risk*		36	27	12			
No. affected Expected		0	0	0			
Observed		0	0	0			

* Sum of affected plus unaffected or unclassified animals under observation up to this age.

unaffected 'carrier' sires and affected dams, 2 (40 per cent) were affected; while of 286 progeny of unaffected sires and unaffected dams, 24 (8·3 per cent) were affected.

The incidence among one age-group of 32 females sired by 3 rams in a flock with a very high incidence throughout the relevant 5 years was 34 per cent. Among the daughters of an affected ram it was 50 per cent, of an unaffected ram 14 per cent and of an unclassified ram 20 per cent.

(7) *Selection.* There is a special bias towards the selection of individuals which have made exceptionally rapid growth in infancy and adolescence with particular development of the skeletal musculature, for such animals tend to win the principal awards at our leading agricultural shows. Many 'show' winners of high excellence have close relatives affected, have affected progeny, or may even manifest the disease later.

(8) *Experimental Transmission.* 5 of 5 lambs inoculated intracerebrally with a brain suspension from a representative affected animal developed a scrapie-like disease 14–24 months thereafter; 5 controls showed no clinical abnormality.

These results are difficult to reconcile with the concept of an infectious disease of the usual communicable type.

The results lend strong support to the view that scrapie is primarily a hereditarily determined disease and establish that the clinical manifestation of the disease is highly heritable.

The results are consistent with the hypothesis that scrapie may be due to a single pair of alleles behaving in a simple Mendelian autosomal recessive manner. It is not possible to rule out other modes of inheritance, notably dominance with variable expression, or to assess the influence of modifying genes and of environmental factors upon manifestation.

As this hereditary scrapie can be transmitted by inoculation, a feature of scrapie amply demonstrated by others[4–6], it is suggested that in the homozygous recessive individual a substance, the transmissible scrapie agent, is formed which is closely associated with the development of the degenerative changes in several organ systems. Transmissible scrapie agent can be demonstrated in many organs[4,5], it is self-replicating[4,5] and its presence can be demonstrated in the experimental disease prior to the onset of clinical signs[5]. It is unusually stable to heat and chemical agents[5,6]. The immunological tests commonly present in virus infections are negative in scrapie[7].

It is postulated that scrapie is a hereditary disease of unusual type, in which the undesirable, indeed lethal, effects of the genotype are mediated through a positive factor, probably chemical in nature, which does not provoke the immune responses usual with self-replicating microbial infections. This concept would go far towards providing a satisfactory explanation of the facts at present known regarding scrapie. Further long-term studies will be necessary to provide adequate data to confirm the hypothesis, to elucidate the role of transmissible scrapie agent in the functional and anatomical pathology of the disease, and indeed to establish whether or not 'scrapie' represents more than one aetiological entity. A detailed report is being published elsewhere.

The generous support of the Nuffield Foundation has made this study possible.

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THE PLEXIFORM ZONE AND SHAPE RECOGNITION IN THE OCTOPUS

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It is the purpose of this article to suggest a possible structural basis for shape recognition in the octopus. The octopus is now the best-investigated animal in respect of its discrimination of visual shape, and this is chiefly due to the work of N. S. Sutherland. This author has also put forward a theory to account for his findings[1], but this theory was criticized by Dodwell[2] on grounds which seem to me to be substantially correct. The cephalopod visual system has also been studied from the neuroanatomical viewpoint by Kopsch[3], Lenhossek[4] and Cajal[5]. The present work is an attempt to infer the way the well-defined neural structures, uncovered chiefly by Cajal, result in the behavioural properties which the octopus displays when discriminating shape.

The System

The basis of the system is an arrangement which converts the distance between two points into an output, the size of which is inversely proportional to the distance. That is, the larger the distance between two imposed points the smaller will be the output, and the smaller the distance between the two points the larger the output.

This operation is performed by an arrangement of the following form. There is a set of lines running parallel to each other. Each line is interrupted at randomly chosen points, so that each line is divided into a number of segments of varying size.

Into this set of parallel segments enter at right angles a set of other lines (to be called retinal receptor fibres). These enter the layer of segmented lines at equally spaced intervals and penetrate the layer of the segmented lines to its other boundary.

Now let us assume that the retinal receptor fibres become active when they have been stimulated by a boundary discontinuity. The distance between two retinal receptor fibres reflects the distance between two points on a boundary. Let us also assume that the line segments in the parallel line layer also become active only when two or more than two simultaneously active retinal receptor fibres cross them. It will then follow that the closer together the two active retinal receptor fibres are, the greater the number of line segments will become active. The sum of active line segments in the parallel line layer will correspond in inverse ratio with the distance apart of the active incoming retinal receptor fibres.

The arrangement which has been described corresponds with what can be seen of the histology of the 'zona plexiforme' of the deep retina as described and illustrated by Cajal[5]. Fig. 1 shows the terminations of the incoming retinal fibres. Fig. 2 shows some of the fibres which may be identified with the segmented parallel line layer of our arrangement. It is to be seen that each of the segments in the section has an output leading out of the 'zona plexiforme' farther into the optic nervous system. We simply have to assume that the fibres in this figure act as a 'coincidence gate' or a spatial summator. If we make this assumption, then arrangements such as these have the function of converting distance between fibres into numbers of impulses. Distances or intervals can then be handled in the same way as intensities. Such a transducer of relational properties does not rely on 'scanning', with its high requirements of timing accuracy and overall precision. Such an arrangement would therefore have implications in the phenomena of neural integration beyond those of cephalopod vision.

So far, the arrangement described would transform the distance between a pair of points into an amount of neural activity The shapes to be discriminated, however, are composed of a great number of pairs of points. It is assumed that these pairs lie on lines parallel to each other and that each pair is measured

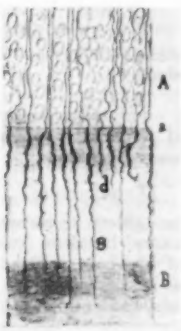

Fig. 1

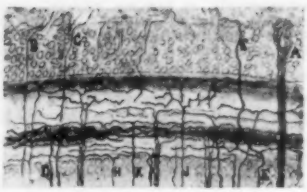

Fig. 2

by an arrangement such as was described above. This is done by having many such arrangements side by side, parallel to each other, running in the vertical visual axis. It is assumed that the total amount of excitation generated by the whole set of these arrangements gives the octopus its chief cue to the discrimination of shape. Different shapes are in this way translated into a single continuum of amount of activity. The problem of discriminating between them becomes one of differentiating between various levels of activity in a single channel.

Before proceeding, I should make clear that for the purposes of prediction two accessory assumptions must be made: (1) that vertical lines produce little or no output, because excitations on the same vertical arrangement inhibit each other over a small distance, and (2) that the octopus can also discriminate relative vertical positions of the output converted in the above way. This second appears to be a residual factor left when we have explained the rest of Sutherland's results. We may assume that it is an independent channel which signals these differences also as differences in quantity of output. It should also be stated that the present system has been left quantitatively flexible. It has been assumed, for example, that the vertical distance between two points leads to an output which is the reciprocal of this distance.

There is no reason for supposing that the conversion is precisely this. The theory only has to assume that the conversion is something like this, and then perhaps not over the whole range (for example, the system may collapse over very large visual angles).

Properties of the System

(1) *Transfer to different sizes of the same shape.* Let us take two parallel lines and their scale enlargement, and place them at right angles to the arrangements described above (that is, in the horizontal visual axis). The total amount of discharge from the system outlined above for the two figures will be the same. If we double the vertical distance between the lines, the amount of firing from each pair of points will halve. However, as we double the distance between the two lines, we also double their length. If we double their length we measure from twice the number of arrangements (as they run parallel to each other in the vertical direction). Therefore, the total amount of output given of both sets of lines separately will be the same. Transfer should therefore be excellent in the case of this shape.

Transfer from one size to another will depend on two factors: (*a*) the shape used; and (*b*) the other member of the pair from which the octopus has learnt to make the discrimination.

As regards the first point, it will be readily seen that, if we take a V shape lying on its side and a scale enlargement of it, the scale enlargement will

produce a greater output. The small V shape is a part of the larger V shape. Such reasoning will apply to diamonds and triangles, *inter alia*. However, any scaling down (or scaling up) will subtract only the pairs of points (or add pairs of points) which are the most distant from each other on the vertical axis. These will be contributing disproportionately little to the total amount of output.

This brings me to the second point. If the total amount of output from the shape to be discriminated from the other member of the pair was larger, then the increase in output due to the enlargement of this shape will not destroy the discrimination, because the effective distance along the continuum is increased. (". . . the octopus, having been trained to discriminate between vertical and oblique, treats the horizontal in transfer tests as equivalent to the oblique (and vice versa for animals initially trained to discriminate between horizontal and oblique)"—Sutherland, 1958. p. 45*b*.) There may actually be an improvement if the other parameters which the octopus derives from the stimulus also increase the distance between discriminanda.

Sutherland has frequently noted that the octopus is capable of maintaining a discrimination between two shapes even when their size is altered (within certain limits) (for example, Sutherland, 1957*a*, p. 65, 1957*b*, p. 13). He has noted that a decrease in size shows a smaller transfer than an increase. This would be predicted on the present theory with certain pairs of figures where the distance along the continuum of output was decreased.

However, a breakdown of the size transfer data according to the shapes to be discriminated has not been considered. A search of Sutherland's data, however, reveals that there is a difference in the predicted direction (Sutherland, 1958). The animals were trained to discriminate between a square and a triangle, both 25 sq. cm. in area. Transfer tests to larger squares (36 sq. cm.) show 65 per cent correct responses. (The accuracy of responding to the original square under the same conditions is 65 per cent.) The figure for transfer to a small square is 67·5 per cent. However, transfer to larger triangles yields a score of 88 per cent in the case of the larger and 67·5 per cent when the triangle is made smaller (changes in area equal to those made in the case of the squares). As a triangle of the same area produces a much larger output than the square, an increase in the size of the triangle will increase the difference in the amount of output between the triangle and the square. Changes in the size of the square will not produce changes in output, if we assume (assumption 1) that its vertical lines produce little or no output. Transfer here should not vary with the direction of the change of size.

The theory is thus in agreement with what is known of the ability to transfer from one size to another in the octopus. Further testing of the theory should examine the relation between shape and the capacity to transfer to other sizes, and also the relation between size transfer and the other member of the pair to be discriminated. We should expect on this theory not only transfer of size to be relative to the other member of the stimuli to be discriminated. Even if the present theory is wrong, it will have been important if it directs attention to these two factors, which so far have been neglected.

(2) *Difficulty of discrimination between shapes.* The difficulty of discrimination on this theory should be

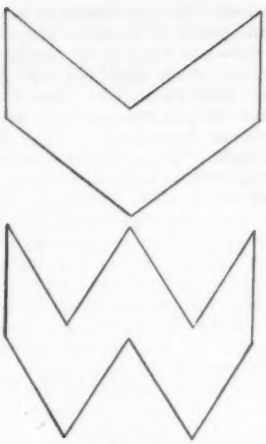

Fig. 3

proportionate to the difference in the quantity of output generated by the two shapes to be discriminated. Each pair of points on the boundary of the shape where it cuts a vertical arrangement will produce an output which will be inversely proportional to the distance of the two points. The sum of the outputs produced by all the pairs of points will characterize the shape for the octopus. The shape, regarding this mechanism only, will be represented as a quantity, or a number on a single dimension. The calculation of similarity can therefore be made with rigour. An index of similarity will be the difference between the two quantities generated by the two shapes to be discriminated. (Such a formulation assumes that the message on all other visual discrimination mechanisms is kept constant, such as that mentioned in assumption 2 (below). There are also others such as brightness, but these are easy to keep constant.)

(1) Sutherland[6] finds that horizontal rectangles are easily discriminated from vertical of the same proportions. This is readily explained. The horizontal rectangle stimulates a large number of vertical arrangements with a set of points relatively close to each other vertically. It is to be remembered that points close to each other produce a larger output than points farther apart. However, the vertical rectangle stimulates a relatively smaller number of vertical arrangements and by pairs of points farther from each other. There should, therefore, be a very large difference (1 : 25) between the outputs if the rectangles are 10 cm. × 2 cm.

"Perfect transfer occurred where the ratio of long to short sides was altered from 10 : 2 to 7 : 2 or 7 : 1" (Sutherland[1]). This would be an alteration at 1 : 25–4 : 49 and 1 : 49 in the amount of output in the discrimination. However, the absolute quantities of output would be different here also. As the direction of the ratios and of the difference remains the same here, this result does not count against the theory and, if anything, supports it. It is a result worth quoting because it shows that in circumstances where the differences among the two discriminanda are large, transfer occurs within a wide margin. It is as if the octopus was simply learning larger–smaller output differences. What would happen if larger numbers of shapes had to be retained in the same experiment by the octopus would be of interest. Further, these

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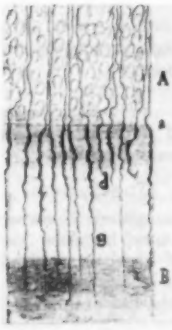

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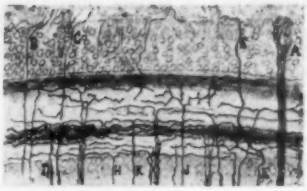

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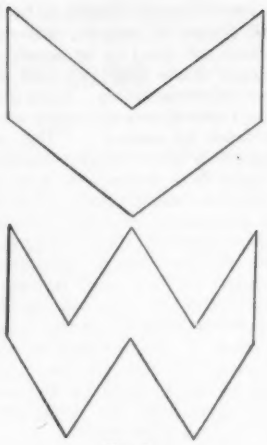

Fig. 3

proportionate to the difference in the quantity of output generated by the two shapes to be discriminated. Each pair of points on the boundary of the shape where it cuts a vertical arrangement will produce an output which will be inversely proportional to the distance of the two points. The sum of the outputs produced by all the pairs of points will characterize the shape for the octopus. The shape, regarding this mechanism only, will be represented as a quantity, or a number on a single dimension. The calculation of similarity can therefore be made with rigour. An index of similarity will be the difference between the two quantities generated by the two shapes to be discriminated. (Such a formulation assumes that the message on all other visual discrimination mechanisms is kept constant, such as that mentioned in assumption 2 (below). There are also others such as brightness, but these are easy to keep constant.)

(1) Sutherland[4] finds that horizontal rectangles are easily discriminated from vertical of the same proportions. This is readily explained. The horizontal rectangle stimulates a large number of vertical arrangements with a set of points relatively close to each other vertically. It is to be remembered that points close to each other produce a larger output than points farther apart. However, the vertical rectangle stimulates a relatively smaller number of vertical arrangements and by pairs of points farther from each other. There should, therefore, be a very large difference (1 : 25) between the outputs if the rectangles are 10 cm. × 2 cm.

"Perfect transfer occurred where the ratio of long to short sides was altered from 10 : 2 to 7 : 2 or 7 : 1" (Sutherland[1]). This would be an alteration at 1 : 25–4 : 49 and 1 : 49 in the amount of output in the discrimination. However, the absolute quantities of output would be different here also. As the direction of the ratios and of the difference remains the same here, this result does not count against the theory and, if anything, supports it. It is a result worth quoting because it shows that in circumstances where the differences among the two discriminanda are large, transfer occurs within a wide margin. It is as if the octopus was simply learning larger–smaller output differences. What would happen if larger numbers of shapes had to be retained in the same experiment by the octopus would be of interest. Further, these

results show that if the two shapes to be discriminated give large differences of output, 'size-constancy' for the octopus does not need to be notably accurate.

(2) Sutherland[6] finds that two oblique rectangles (/ and \) are indiscriminable. Here the sums of the outputs in the vertical arrangements are the same.

(3) Furthermore he stated[6]: "The finding that a vertical rectangle is more readily discriminable from an oblique than is a horizontal was unexpected". Such a difference is readily predicted from the present theory. The horizontal rectangle would produce a much greater output than the vertical rectangle. The output of the oblique rectangle would not be intermediate as might at first sight appear because the tilt of the figure would produce a set of points close to each other at the corners on the vertical axis. It is predicted that if the corners of the horizontal figure were altered to make it into a parallelogram with the short sides parallel to the vertical visual axis (the area being kept the same) the differential discrimination would disappear.

(4) Sutherland[7] has found that V and W shapes (Fig. 3) of the same height, width and area can be discriminated with great difficulty or not at all (accuracy 53 per cent) when the figures are in this orientation. An inability would be predicted on the present theory. The total vertical distances are the same in both cases. However, it would be predicted that the same shapes would be more discriminable when both are tilted through 90°. With $\lesssim$ the vertical distance is divided into more short segments than in the case of $<$. Therefore, as short segments produce more output than long, and there are here more short segments, output should be larger for $\lesssim$ than $<$.

(5) Sutherland[7] has found that $\vee$ is indiscriminable from $\wedge$ (accuracy 50 per cent). This can be predicted in a straightforward way. He has also found that $<$ can be discriminated from $\wedge$ (though only with an accuracy of 56 per cent). The $<$ shape would cut the vertical arrangements at points closer to each other than the $\wedge$ shape, thus producing more excitation. However, $\wedge\!\wedge$ is discriminated from $>$ with greater ease (69 per cent accuracy) and the present theory cannot readily account for this or the quantitatively small success of the octopus with $<$ against $\wedge$. (There are, however, assumptions about the sensitiveness to alterations in output with equal inaccuracies of verticality which differ for the shapes used and the amount of the shape deleted due to assumption 1 which could be made.)

(6) Sutherland (1958*a*) has found that octopuses have more difficulty in discriminating a diamond (a square with base at 45° to the horizontal) from a triangle than in discriminating a normal square from a triangle. This again is in accordance with the calculations of differences of magnitude of output derived from the theory. Though the other findings on the discriminability of the standard geometrical shapes (circles, triangles, etc.) show good agreement with the theory, differences in height (assumption 2) equally well account for some of the findings, as shapes of equal area were used. A further point to be borne in mind is that so far the assumption has usually been made that the placing of the shape on the receptor field of the octopus relative to the vertical has been geometrically exact, and departures from this have not been considered. However, the output of some shapes is much more affected by deviations. It is possible that the octopus uses this magnitude of oscillation about a mean to effect some discrimination. This supposition could be tested by imposing known and differing degrees of rotation in one direction and then the other in quick succession on the same shape, one amount serving as the positive signal and the other as the negative.

(7) It is found that the octopus can discriminate some up–down mirror images. This would be covered by assumption 2. However, left–right mirror images are indiscriminable, and this agrees with the theory.

Discussion

In the previous section, the salient reported findings on the shape discrimination of the octopus have been discussed in relation to the proposed system. The reader must be the judge of the success of the system in accounting for these findings. Predictions have also been made which allow the theory to be tested behaviourally.

However, the theory is not simply behavioural, and in this sense it is of much wider scope. A functional significance is suggested for a neuronal network of a kind to be found in many other places. It is proposed that this network converts relative distance into an output simply of quantity, and a simple *modus operandi* is given. In this way a look at the neurohistology may give a clue to the component operations on information in neural systems the overall function of which is already known. Direct work correlating visual input with events in the cephalopod optic lobes can also be used to check the correctness of my surmise.

On a more general plane it is conjectured that the octopus breaks down its visual information into various 'components' or dimensions of which the system put forward is only one. Each of these 'components' (given varying visual input) will vary only in amount. This amount for each 'component' is presumably represented as a quantity of excitation leaving the optic lobes, via one of the nerve fascicules. If this is correct, then sectioning one of these bundles, while conducting behavioural tests, would reveal the kind of classification or breakdown of the visual environment which the octopus performs by noting resulting confusions. Similarly, recording from these nerve bundles (should this prove feasible) while the eye is stimulated with various inputs should prove revealing. It is indeed possible that the transformation of shape into a simple quantitative continuum may prove to be a paradigm of the way that the problem of perceptual classification is dealt with in other organisms. In this way a given environment could be translated into a set of levels of activity in a number of channels. Thus a state of affairs for the organism could be represented as a point in an n-dimensional space.

My thanks are due to Prof. T. H. Bullock for discussion and encouragement, to Prof. J. Z. Young for helpful correspondence and neuroanatomical data, to Dr. N. S. Sutherland for discussion of his results, and to Dr. G. A. Horridge for helpful comment.

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LETTERS TO THE EDITORS

GEOMORPHOLOGY

Bedrock Corrosion and Drainage Initiation by Seepage Moisture on a Gritstone Escarpment in Derbyshire

A SURVEY has been made of the podsolized soils, the morphology and the drainage of the areas known as Matlock Moor, Farley Moor, Tower Moor and Blackbrook Moor, which together form the larger part of the upper reaches or 'source basin' of the Bentley Brook, a tributary of the Derbyshire Derwent[1].

Observations have been made which show the corrosive action upon the underlying bedrock of water seeping through a 2-ft. deep layer of sandy colluvial material occupying a seepage hollow at the head of the brook. Such water, slow-moving, and with an appreciable content of humus, seeping vertically on to the bedrock, has corroded it to depths of 18–22 in. The layers below 18 in. in the whole profile are permanently wet and have been so even in the dry summer of 1959. The upper mineral layers of the profile are lacking in particles finer than 0·06 mm.; but it is not yet certain whether such particles are removed by normal vertical seepage to be redeposited at lower levels, or are removed by lateral movement of soil water, near the surface, in the wettest periods of the year.

Other observations provide evidence of the processes associated with the initiation of a semi-dendritic drainage pattern on a dip-slope, of less than 5°, adjacent to the hollow, and on the scarp slope to the west, overlooking the Sydnope valley. Integrated narrow seepage lines express themselves as overdeepenings of the solum upon such slopes, though there is no surface indication of their presence. A close grid of augerings across and along them shows significant changes of soil profile morphology both within the seepage lines and on the areas between them. These seepage lines link up with visible surface drainage at lower levels.

These two types of seepage action which affect the bedrock on the watershed between opposing dip and scarp streams do not suffice to break the crest of the escarpment. As a result of their headward extension, the seepage catchment of the watershed area becomes so small that the processes of corrosion and overdeepening can no longer continue except at some point downslope where enough moisture can assemble. Hence the crest is exhumed to form a rock platform on which tors may develop wherever the wide spacing of the master joints permits their survival.

The observation that a rock platform on the watershed between two opposing drainage systems may not only be preserved, but may actually broaden by exhumation, calls for a reappraisal of the current ideas on col-formation in the normal cycle of erosion, at least on uniformly hard rocks. It also leads to a hypothesis of tor formation applicable to some of the tors of the southern Pennines. The tors in question are formed during the gradual emergence of crestal rock platforms from adjacent bands of corroded bedrock and colluvial material. These successive bands form Penck's convex and intermediate slopes[2], although they are produced by seepage, not by drainage in visible channels.

The colluvium merges into a band of thin soils which then merges downslope into a mass of valley-head peat wherein the permanent source of integrated drainage is found. The peat often rests upon the rock identical with that of the tor. The rock is pierced in a pronounced knickpoint by the stream, which flows down over lower gritstone beds and intervening partings of shale before entering the wide main valley which is cut in the dominantly shaly rocks below the lowest grit and above the limestone.

There exists a possibility of assessing the period of time necessary to exhume these tor features. Most of them are 14–16 ft. in height. Young's estimate of surface lowering of the whole area of a small drainage basin, 17 miles to the north of the area considered here, is 0·005 in. per annum[3]. If one assumes a height of 15 ft. and that the tops of the tor features have not been lowered by weathering, then the time at which they began to be exhumed should be 36,000 years ago—a date which closely corresponds to the waning of the York glacier[4].

A more detailed account of this work will appear elsewhere. My thanks are due to Dr. G. Dury and Mr. R. A. G. Savigear for their comments and advice. This work has been supported in part by a grant from the Central Research Fund of the University of London.

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GEOLOGY

Mid-Tertiary Stratigraphical Palæontology

IN the course of extensive economic palæontological researches based on world-wide investigations, it has been found necessary to review and re-assess the bases of mid-Tertiary correlation and dating. We confirm the validity of the five-fold subdivision of the Oligocene and Lower Miocene as represented by the faunas of the type localities of the Lattorfian, Rupelian, Chattian, Aquitanian and Burdigalian. These five faunal assemblages can readily be correlated through to Southern Europe, the Mediterranean, the Middle East, East Africa, Pakistan and India, and the Far East as a whole.

Although the classical type assemblages are mainly developed in a molluscan–echinoid facies we confirm that it is possible to correlate these facies with beds in Southern Europe, which also contain important and characteristic foraminiferal faunas which can readily be followed through to the Far East, notwith-

standing that minor stratigraphical and taxonomic revisions of older work are necessary.

In brief, beds equivalent to the Lattorfian are characterized by *Nummulites intermedius* and *N. vascus* without *Eulepidina*; the equivalents of the Rupelian are characterized by the persistence of *Nummulites intermedius* and *N. vascus* and the appearance of *Eulepidina*; the equivalents of the Chattian (where confirmed) are characterized by the persistence of *Eulepidina* and other Rupelian Foraminifera, but all true *Nummulites* had become extinct. Following the mid-Tertiary stratigraphical break, the equivalents of the Aquitanian (forming the lowest beds of the Miocene) are characterized by the persistence of *Eulepidina*, *Spiroclypeus* and *Miogypsinella complanata*, and the incoming of many forms, such as *Miogypsina* s. str., *Meandropsina anahensis* and *Miolepidocyclina*, which constitute part of the Neogene evolutionary surge; the Burdigalian equivalents are characterized by such forms as *Ostrea latimarginata*, *Borelis melo curdica*, *Flosculinella bontangensis* and *Taberina malabarica*, by the persistence of *Miogypsina* and *Austrotrillina howchini*, and by the appearance of *Borelis melo*, but *Eulepidina* and *Spiroclypeus* had become extinct.

It can be evaluated from the above that in the Far East the '*c*' stage is probably Lattorfian, the '*d*' stage is Rupelian, the '**e**' stage is Aquitanian, and the 'f_{1-2}' substages are Burdigalian; the presence of the Chattian is not proved. Besides the fossils mentioned above there are many others which substantiate the above conclusions.

Within the equivalents of the five classical stages there is a succession of planktonic foraminiferal faunas (Globigerinaceae) which appears to be constant wherever found and studied. These planktonic successions can be firmly dated in terms of the classical stages by their associated faunas; they permit accurate age determination of marine faunas in the western hemisphere, where, because of relative isolation, few benthonic fossils are in common with their Eurasian and African equivalents.

It has been possible to demonstrate the equivalence of the Caribbean zones of *Globorotalia fohsi fohsi*, *G. fohsi lobata* and *G. fohsi robusta* to the Burdigalian. The Aquitanian seems to be equivalent to all the planktonic foraminiferal zones erected by Bolli (1957) for the Cipero formation of Trinidad below the *G. fohsi fohsi* zone; consequently, all the Vicksburgian and its equivalents are Aquitanian. The evolution of *Orbulina*, known to occur in the upper part of the *Globigerinatella insueta* zone of the Caribbean, also occurs in the highest Aquitanian of the Mediterranean and Australia; this correlates with the first appearance of *Orbulina* in the upper '*e*' stage of the Far East.

It seems to be very important to note that, on the basis of the succession of planktonic foraminiferal faunas, no Oligocene can be recognized in the marine sediments of the western hemisphere, as far as published records show. This absence of Oligocene has now been confirmed by the discovery of a planktonic foraminiferal fauna associated with true Oligocene larger foraminifera such as *Nummulites intermedius* and *N. vascus* in East Africa. This planktonic foraminiferal fauna is being described elsewhere; it is noted here as having common elements with similar faunas seen in the Rupelian of south Germany and that described by Russian workers. This fauna has never been recorded or observed by us in Central American areas, and is missing precisely where we place the major stratigraphical break in that region.

The absence of marine Oligocene in the Central American region is again confirmed by the presence of *Miogypsina* s. str. associated with *Eulepidina* and of *Pliolepidina* in the lowest post-Eocene beds of the well-known southern Trinidad succession, which contains the oldest known post-Eocene planktonic zone of the Caribbean. True *Nummulites* (as distinct from *Palæonummulites*) is absent above the Eocene in the Caribbean.

Palæonummulites Schubert 1908 (type species *Nummulina pristina* Brady 1874) is regarded as a prior synonym of both *Operculinella* Yabe 1918 and *Operculinoides* Hanzawa 1935. The lectotype of *Palæonummulites pristina* (Brady) has been isolated and it will be described elsewhere, together with an emendation of the genus.

The genus *Pliolepidina* H. Douvillé 1915 is now regarded as a prior synonym of *Multicyclina* Cushman 1918, *Multilepidina* Hanzawa 1932, *Cyclolepidina* Whipple 1934, and *Pliorbitoina* Van de Geyn and Van der Vlerk 1935. It is believed that all species referable to this genus, whether originally recorded from the Far East, the Mediterranean region, East Africa, or Central America, are of Miocene age. The large and significant unconformity present in the Central American region is marked by the presence of much re-working of material into the basal Miocene and this has given rise to much stratigraphical confusion as to the age of the beds in question. Indeed, many of the lowest Miocene beds present have been dated as Eocene, purely on the presence of re-worked Eocene fossils, but the occurrence of *Pliolepidina* often shows that they are of lowest Neogene age; examples are the San Fernando formation of Trinidad and part, at least, of the Gatuncillo formation of Panama.

Full details of our work will be published in the near future elsewhere, together with a full bibliography.

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Oriented Quartz Inclusions in Garnets

Metamorphic garnets in the Central Highlands of Scotland frequently contain oriented inclusions of quartz, which throw light on the mechanism of deformation of the latter mineral. In particular, garnets which have an S-shape arrangement of inclusions and have consequently grown at the same time as deformation show a variety of morphogenetic types of quartz grains, which were incorporated into garnet as inclusions.

In a specimen of mica-amphibole-garnet schist from the River Tummel (National Grid No. *NN.* 899605) there are three types of such grains. In the centre of the garnet there is a number of crescentic inclusions (about 0·05 mm. × 0·01 mm. in size), which have no visible strain shadows, glide lamellæ or deformation bands, but which in cross-polarized light

are nevertheless optically inhomogeneous, although even at high magnifications the constituent units responsible for the inhomogeneity cannot be recognized. Associated with these grains, but continuing towards the periphery of the garnet, there are much larger elongated quartz inclusions (about 0·15 mm. × 0·1 mm. in size) which are optically homogeneous and are not crescentic in shape. Lastly, at the periphery of the garnet there are relatively equidimensional grains, again much larger than the first variety (0·1–0·15 mm. across), and which may have strain shadows when the grains are continuous into the matrix.

In the crescentic inclusions the average optic axis of the constituent quartz is normally slightly oblique to the elongation of the inclusions, while in the elongated optically homogeneous variety the optic axis tends to be parallel to the elongation of the grain. In the peripheral grains no constant relationship can be established between the position of the optic axes and the dimensions of the inclusions. The S-shape trend of inclusions implies that the innermost grains have been included into the garnet early in the process of movement, while the outermost individuals record the immediately post-movement stage of lattice re-organization. It is clear that throughout the deformation the fabric of the rock coarsened progressively and that the early dimensionally oriented fabric is conditioned by movement, whereas the later fabric did not develop dimensional orientation, and was presumably formed under static conditions. Since at an earlier stage the sites now occupied by the dimensionally unorientated fabric must have had quartz grains like those preserved in the interior of the garnet, the peripheral quartz has suffered severe re-organization.

Fellows[1] recognized that deformed crystalline quartz undergoes first recrystallization and then neo-crystallization. In the garnet described, the crescentic inclusions reflect the initial deformation, while the elongated optically homogeneous grains have recrystallized. These grains are analogous to mimetic mica flakes, and those in the matrix, according to this interpretation, are neo-crystalline. Both recrystallization and neo-crystallization are recognized in metallurgy by Burke and Turnbull[2]. The deformed crescentic grains can be interpreted as polygonized grains (cf. Cahn[3]) retaining their initial fine deformation texture, which is not resolved under a microscope. On the other hand, the strain-shadows in the peripheral grains must have originated during an episode of renewed deformation which succeeded the neo-crystallization.

The fact that there are slight differences—in the relationships of the optical orientation and elongation—between the crescentic (polygonized) and recrystallized grains suggests that recrystallization affects the overall lattice orientation, and that this factor must be considered when the behaviour of metamorphic quartz is studied.

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PHYSICS

A Theory of Ball Lightning Formation

In a paper which has recently attracted a considerable amount of attention, Kapitza[1] has proposed a standing electromagnetic wave description of ball lightning. His paper presents a good qualitative explanation of this effect in terms of the resonance absorption of a standing wave due to a conducting ionized sphere of plasma, occurring when the wavelength of the radiation is approximately four times the diameter of the fireball.

It is the purpose of this communication to point out that a polarized electromagnetic standing wave can actually produce containment of charged particles in the neighbourhood of its electric nodes, and that this effect should be important in the initial stages of fireball formation. As a result of this investigation it has been shown that it is possible for electrons to acquire sufficient energy in the electric node regions to produce further ionization by collision, provided that the standing electromagnetic wave yield-strength is in the vicinity of 10^6 V./m. At first sight this figure may seem unusually high; nevertheless it should be recalled that field strengths of this magnitude are already required to cause atmospheric breakdown during thunderstorms. It is proposed, therefore, that the atmosphere is initially very weakly ionized (due to the thunderstorm) and that the regions around the electric nodes rapidly become highly ionized due to electron ionization by collision, building up in an avalanche fashion until the plasma frequency ω_p is greater than the frequency of the applied field ω. At this point further field penetration of plasma becomes impossible, so that surface absorption of energy becomes responsible for maintaining the fireball in a manner similar to that suggested by Kapitza. Moreover, the spherical shape of fireballs fits into the ultimate surface absorption picture quite well, since this is a direct consequence of the fact that the configuration of minimum surface energy requires a spherical distribution.

The actual containment of a charged particle in a polarized standing electromagnetic wave will be demonstrated using a straightforward technique similar to that developed by R. Wuerker (unpublished private communication) and Cushing and Sodha[2].

We shall assume that a polarized standing electromagnetic wave can be represented by

$$B = B_0 \sin \omega t \cos \frac{2\pi x}{\lambda} \hat{k} \qquad (1)$$

$$E = -cB_0 \cos \omega t \sin \frac{2\pi x}{\lambda} \hat{j} \qquad (2)$$

where B_0 represents the maximum value of oscillating field of frequency ω, and wave-length λ, and $\hat{i}$, $\hat{j}$, $\hat{k}$ are the usual cartesian unit vectors.

The following equations of motion of a charged particle may be derived using equations (1) and (2) and the Lorentz force equation:

$$\ddot{x} = \frac{eB_0}{m} \dot{y} \sin \omega t \cos \frac{2\pi x}{\lambda} \qquad (3)$$

$$\ddot{y} = -c \frac{eB_0}{m} \cos \omega t \sin \frac{2\pi x}{\lambda} - \frac{eB_0}{m} \dot{x} \sin \omega t \cos \frac{2\pi x}{\lambda} \qquad (4)$$

$$\ddot{z} = 0 \qquad (5$$

Integrating equation (4) we immediately obtain :

$$\dot{y} = -\frac{c}{\omega}\frac{eB_0}{m}\sin\omega t \sin\frac{2\pi x}{\lambda} + C \qquad (6)$$

where the constant C represents the initial velocity of the particle in the y-direction at time $t = 0$.

Direct substitution of equation (6) into (3) gives :

$$\ddot{x} + \frac{c\omega}{4}\left(\frac{eB_0}{m\omega}\right)^2\left\{1 - \cos 2\omega t\right\}\left\{\sin\frac{4\pi x}{\lambda}\right\} = \frac{eB_0}{m}C\sin\omega t\cos\frac{2\pi x}{\lambda} \qquad (7)$$

Equation (7) is clearly a difficult equation to solve ; however, if we concern ourselves with solutions of (7) representing oscillations of small amplitude about the electric field nodes, we may write :

$$\cos\frac{2\pi x}{\lambda} \simeq 1 \qquad (8a)$$

$$\sin\frac{4\pi x}{\lambda} \simeq \frac{4\pi x}{\lambda} \qquad (8b)$$

and equation (7) can be reduced to the form :

$$\ddot{x} + \frac{1}{2}\left(\frac{eB_0}{m}\right)^2\left\{1 - \cos 2\omega t\right\}x = \frac{eB_0}{m}C\sin\omega t \qquad (9)$$

which is in the form of a driven Mathieu equation. The solutions of the Mathieu equation have bound or unbound characteristics depending upon the choice of parameters[3]. For example, when :

$$\frac{1}{2}\left(\frac{eB_0}{m\omega}\right)^2 < 0{\cdot}65 \qquad (10)$$

the system operates in the first region of stability, and the solution of equation (9) takes the following bound form :

$$x = F\left\{\cos\omega_0 t - \frac{a}{8}(1+\sqrt{a})\cos(2\omega-\omega_0)t - \frac{a}{8}(1-\sqrt{a})\cos(2\omega+\omega_0)t + \ldots\right\} + G\left\{\sin\omega_0 t - \frac{a}{8}(1+\sqrt{a})\sin(2\omega-\omega_0)t - \frac{a}{8}(1-\sqrt{a})\sin(2\omega+\omega_0)t + \ldots\right\} \qquad (11)$$

where F and G are constants of integration and ω_0 represents the oscillating frequency of the particle about the electric node along the x-axis. Where we have defined :

$$a = \frac{1}{2}\left(\frac{eB_0}{m\omega}\right)^2 \qquad (12)$$

and

$$\omega_0 = \frac{eB_0}{\sqrt{2}m}\sqrt{1 + \left(\frac{eB_0}{4m\omega_0}\right)^2} \qquad (13)$$

It is convenient to represent the harmonic binding of the particle about the node by means of an effective potential. In the first region of stability this assumes the form :

$$V(x) = \frac{1}{2}\left(\frac{m}{e}\right)\omega_0^2 x^2 \text{ volts} \qquad (14)$$

By the substitution of condition (10) into equation (6) it is possible to find the y-motion about the electric nodes, and it has been shown that the charged particle is quasi-bound in this direction. The particle will describe Lissajous's figures in the xy plane, with dominant frequencies of ω_0 and $(\omega-\omega_0)$ in the x and y directions, respectively. Having convinced ourselves that a charged particle can be bound in the vicinity of an electric node, it will suffice to say that a corresponding investigation of solutions in the neighbourhood of the electric anti-nodes yields no bound solutions ; in fact, indicates that the particles are pushed away from the anti-nodes toward the nodes. Although a charged particle is essentially contained in the x–y plane, it is clear that there is no binding in the z-direction. Nevertheless the x–y binding will allow the particle to be 'contained' in such a manner as to allow it to produce considerably more ionization by collision before drifting out along the z-axis, ultimately leading to a region of high plasma density and conductivity in the electric node neighbourhood. On the basis of Kapitza's arguments, standing wave frequencies in the neighbourhood of 10^9 cm./sec. would be required to explain the 'observed' fireball sizes of 10–20 cm. Putting this value of ω into condition (10) along with the required electric field strength of 10^6 V./m., it can be seen that the condition for containment in the first region is easily satisfied. In addition, Kapitza has suggested that a fireball is located at the electric field anti-node, whereas our description indicates that the formation process would be expected to take place at the electric node.

Finally, it is suggested that this theory could be tested under laboratory conditions. This could be accomplished by using a standing wave created by a pair of travelling waves fed from opposite sides of the containment region. This is necessary in order to preserve the symmetry about the y–z plane, after the plasma conductivity has reached a point where surface absorption is predominant. Theoretical investigation of genuine three-dimensional plasma containment is at present in progress using three separate standing waves parallel to the x, y and z axes, respectively, the results of which will be published later elsewhere.

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Neutron Diffraction Study of Antiferromagnetic Spin Waves in α-Ferric Oxide

It has been shown by Elliott and Lowde[1] that spin waves will manifest themselves in the neutron scattering by a crystal as coherent diffuse peaks near the elastic reflexions. In particular, the dispersion relation of the spin waves may be directly obtained from the way the diffuse peak-width Γ changes with $\theta - \theta_B$, the angular missetting of the crystal from the Bragg setting. Such measurements have been done on crystals of iron[2] and magnetite[3], and in both cases a quadratic dispersion law was found.

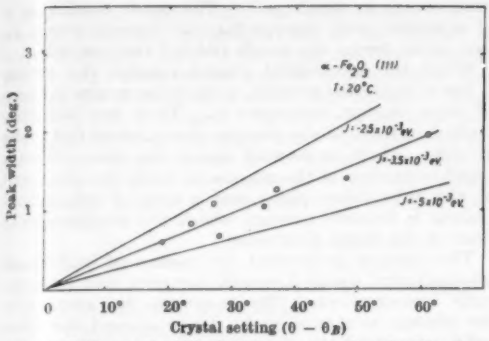

Fig. 1. Diffuse peak width plotted versus angular missetting from Bragg position for (111) reflexion. Straight lines have been calculated for different spin-wave velocities, corresponding to the indicated values of the predominant exchange integral

It may be shown on general grounds that in an antiferromagnet the dispersion law for large wavelengths is linear, so that there is a magnon propagation velocity c_m independent of frequency. As a consequence the relation between Γ and $\theta - \theta_B$ must also be expected to be linear.

Measurements of diffuse peak widths of antiferromagnetic α-Fe_2O_3 (hematite) near the (111) reflexion (rhombohedral indices) indeed show this linear dependence (Fig. 1). From the slope a spin-wave velocity $c_m = 3{\cdot}76 \times 10^6$ cm./sec. is deduced.

A theoretical expression for the spin-wave velocity may be found by solving the secular equation arising from a Heisenberg-type exchange coupling between the spins of the iron atoms, which in hematite belong to four different sub-lattices. The expression contains atomic position co-ordinates and the exchange integrals. The latter have been discussed by Gilleo[4] in terms of super-exchange interactions between iron spins through intervening oxygen atoms. These interactions turn out to be of four types, differing in interatomic distances and angles, and with different values of the exchange integral J. If now, following Gilleo, one ignores all interactions except those belonging to the type with the largest $|J|$, the expression for the spin-wave velocity becomes:

$$c_m = -2^{5/2} JSa/\hbar \qquad (1)$$

where S is the iron spin and $a = 5{\cdot}03$ Å. the base of the hexagonal unit cell. The value of c_m is actually slightly dependent on direction of propagation, but this anisotropy is within 4 per cent. Substituting the observed c_m in (1) one finds for the predominant exchange integral $J = -0{\cdot}0035$ eV., the minus sign indicating an antiferromagnetic coupling.

The value of the exchange integral thus found may be used in a calculation of the diffuse scattering cross-section. Elliott and Lowde[1] have given an expression for the cross-section integrated over the whole diffuse peak for a simple class of antiferromagnets and shown that it is independent of crystal missetting. Their treatment may be generalized to cases including that of hematite for which, with the above value of the exchange integral, it leads to a constant cross-section of 1·4 millibarns per iron atom. The observed values are plotted in Fig. 2 for different angles of misset and show this general behaviour, although with a somewhat higher cross-section value of 1·7 millibarns.

It remains to prove that the observed diffuse peaks are indeed due to antiferromagnetic spin waves and not to lattice vibrations, which also would show a linear dispersion relation. Since the nuclear structure factor of the (111) reflexion is zero, such vibrations could only manifest themselves as magnetic vibrational scattering. To distinguish between the two kinds of scattering one may use their different dependence on the direction of spin alignment. The spin wave cross-section contains a factor $(1 + e_z^2)$, where e_z is the cosine of the angle between the spin direction and the normal to the reflecting plane. The magnetic vibrational scattering, on the other hand, is proportional to $(1 - e_z^2)$, as is the elastic magnetic scattering. This effect has been used in the study of ferromagnetic spin waves, where e_z may easily be varied by an external magnetic field.

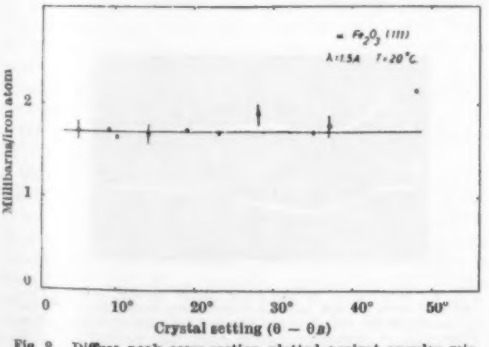

Fig. 2. Diffuse peak cross-section plotted against angular missetting

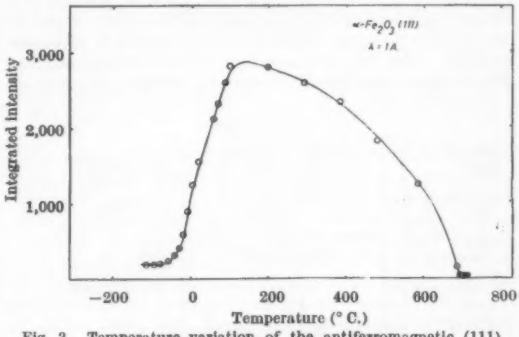

Fig. 3. Temperature variation of the antiferromagnetic (111) Bragg reflexion

In the case of antiferromagnetic spin waves this possibility does not exist. However, in hematite the spin direction is dependent on temperature. Fig. 3 shows the temperature variation of the elastic scattering in the (111) Bragg peak. It is in agreement with the observation of Shull *et al.*[5] that at low temperatures the magnetic moments are pointing along the [111]-direction ($e_z = 1$), whereas at high temperature they are in the (111)-plane ($e_z = 0$). From Fig. 3 and the fact that the inelastic scattering is proportional to absolute temperature, it may be inferred that when the crystal is cooled from 20° C. to − 35° C. the intensities due to spin waves and lattice vibrations should change by + 12 per cent and − 92 per cent respectively. Experimentally an increase of 10 ± 5 per cent was obtained, showing that the diffuse peaks must be of almost pure spin-wave origin.

A complete account of this work will be published elsewhere.

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Experiments with Plasma Rings

The use of a coaxial plasma gun with radial magnetic field for the production of magnetized plasma rings was suggested[1] by H. Alfvén in 1958. Experiments with a plasma gun of that type were started in the spring of 1958 and a detailed report is to be published[2]. We present here some new results obtained during continued experiments with a slightly modified plasma gun.

A detailed description of the equipment is given in the previous paper[2]. The main features are described here with reference to Fig. 1, which shows a cross-section of the apparatus. Between the coaxial electrodes of the plasma gun a discharge is set up in hydrogen with a pressure in the range of 0·03–3 torr. The energy, initially stored in the condenser bank of 200 μF., charged to 5–8 kV., is released when the spark gap is triggered by a high-voltage pulse. The electrodynamic force in the plasma pushes the ionized gas towards the muzzle of the gun. Here it encounters a static magnetic field, which is directed radially. The field is maintained by an electromagnet. The central conductor of the gun, which is made of iron, serves as north pole. The outer conductor is of stainless steel, except for the muzzle where an iron plate forms the south pole of the magnet.

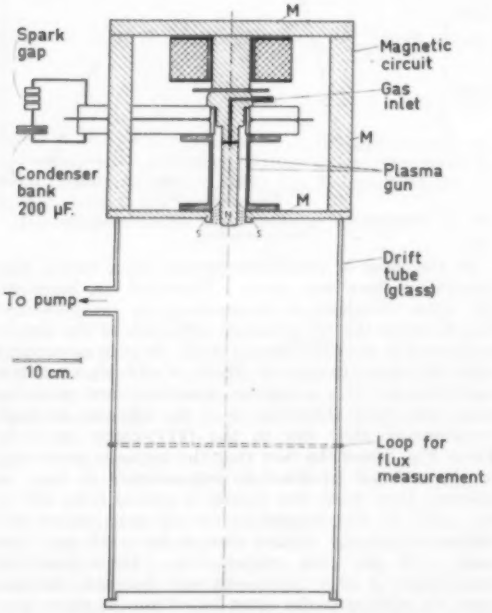

Fig. 1. Cross-section of plasma gun with drift tube

When the accelerated plasma reaches the muzzle it has a high conductivity, so that the magnetic lines of force cannot penetrate it. They are therefore pushed in front of the plasma, down into a drift tube of glass, which is located under the gun. Here a rapid expansion of the plasma towards the glass wall of the tube takes place and a ring of magnetized plasma is formed, through which the stretched field lines of the static field close.

The plasma is studied by means of high-speed photography, using Kerr-cell cameras, and by magnetic measurements. The magnetic flux carried by the plasma is measured by loops around the glass tube connected to integrating resistance-capacity circuits ($R = 10$ k.ohm, $C = 0{\cdot}05$ μF.).

Fig. 2 shows a typical picture of the discharge taken 4 μsec. after the ignition. The luminous front, which probably is due to a shock wave preceding the magnetized plasma, reaches the bottom of the drift tube at about 9 μsec.

Fig. 2. Kerr-cell photography of the discharge taken 4 μsec. after the ignition

Fig. 3 shows oscillograms of the circuit current and the magnetic flux measured by a loop situated 40 cm. below the muzzle of the gun. The circuit current performs a strongly damped oscillation with a peak value in the first half-cycle of 220 k.amp. and passes through zero 12 μsec. after the ignition. The magnetic flux increases to a maximum, which often appears when the circuit current passes through zero, and then decays approximately exponentially. As the circuit current passes through zero the flux curve is smooth and the direction of the flux through the loop does not change. This indicates that a current continues to circulate in the plasma independently of the circuit current, which is rapidly damped out. Even at 40 μsec. after ignition, a toroidal current is circulating in the plasma.

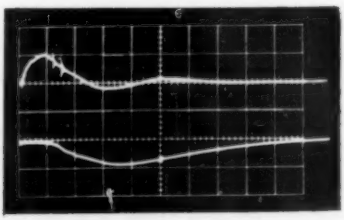

Fig. 3. Oscillogram of the main circuit current (upper curve) and the magnetic flux measured by a loop around the drift tube 40 cm. below the muzzle of the gun and recorded after integration. Time-scale, 5 μsec./div., current scale, 220 k.amp./div. Flux scale, 1 mWb./div. The applied static flux in the plasma gun was 0·47 mWb.

One would expect that the ring-shaped plasma passing the static magnetic field at the muzzle of the gun would conserve the magnetic flux through the central conductor of the gun as a 'poloidal' flux through the hole of the plasma ring formed. Often, however, the flux carried by the plasma is several times greater than the static flux. Whether this can be ascribed to asymmetry in the discharge or whether it depends on some mechanism by which the toroidal flux in the ring is transformed to a 'poloidal' flux is not clear.

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CRYSTALLOGRAPHY

Spiral Growth of Colloidal Gold and Moiré Fringe

Many examples of spiral growth have been reported since Frank[1] introduced the idea of screw dislocation into the general mechanism of crystal growth. Amelinckx[2] has reported growth spirals which appeared on chemically deposited crystals of gold. We also wish to report various growth spirals and related matters recently observed by electron microscopy on lamellar single crystals of gold, prepared colloid-chemically under definite conditions as discussed in previous papers[3]. Brüche and Demny[4] also reported similar patterns on gold crystals and discussed the growth mechanism.

Fig. 1*A* shows a typical example of a lamellar single crystal of colloidal gold having the growth spiral. A small hole exists at the centre of the spiral, from where we can follow the ridge line and, after passing on the outer perimeter of the crystal, we can come back to the initial starting point. The three-dimensional configuration of such a crystal is suggested by Fig. 1*C*, which is an electron micrograph of a specimen replicated by carbon film *in vacuo*, to be in a manner demonstrated by Fig. 1*G*, though slightly exaggerated in its step height. The two spirals on both surfaces of a crystal are nearly symmetrical to each other in most of the present examples. This seems very reasonable as the specimens were grown in a suspension and the condition of the medium was quite the same on both sides of each crystal during growth. Fig. 1*B* shows another example where a rather regular hexagonal loop appeared. We can also observe a small hole as well as the returning loop as indicated by arrows.

One of the most outstanding features in the present specimens is that sharp moiré fringes appeared on the growth steps when observed under an electron microscope. Various causes for the moiré fringe have been reported so far[5]. For the present case, we think it reasonable that it is mainly caused by the slight azimuthal rotation of each step, which takes place against another step on which the former one lies and around an axis parallel to the Burgers vector of the screw dislocation, which in turn is normal to the

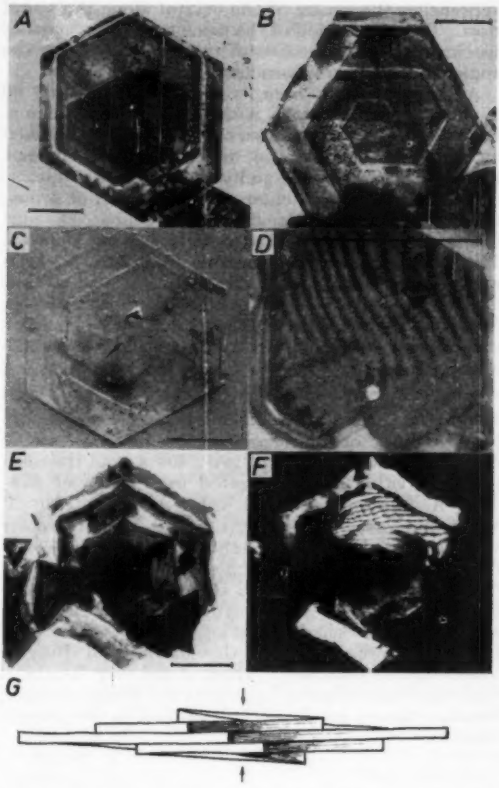

Fig. 1. Electron micrographs and schematic demonstration of single crystals of colloidal gold with growth spirals. Each scale gives 1μ. In Fig. 1*G*, the two arrows show the position of a core of screw dislocations

flaky (111) plane of the crystal. Neither considerable separation nor relative inclination of these steps was detected so far as the methods of shadow casting and carbon replica were concerned. We have also taken a set of dark-field images of moiré fringes formed with the electron beam reflected by the lattice plane contained in the region of the crystal where two or more steps were superposed. Fig. 1*F* shows an example of these dark-field images formed with the $(2\bar{2}0)$ reflexion of the crystal, the ordinary electron micrograph of which is shown in Fig. 1*E*. In the dark-field image, there appeared nearly parallel thick moiré fringes, the direction of which is again parallel to $[2\bar{2}0]$ axis of the crystal. This means that they were formed by the interaction of two electron beams reflected respectively by each $(2\bar{2}0)$ plane contained in two superposed steps. The moiré fringe is certainly very sensitive to the slight distortion of the crystal. But when the cause of the fringe is supposed to be attributable only to the slight azimuthal rotation of steps, the angle of rotation α is given by an equation[6]: $\alpha = d_{2\bar{2}0}/D$, where D and $d_{2\bar{2}0}$ are the spacing of the moiré fringe and the interplanar spacing of the $(2\bar{2}0)$ plane. With the set of dark-field images, the angle α has been estimated to be of the order of 2×10^{-3} rad. on an average. Further, it increases roughly linearly from 7×10^{-4} rad. to 4×10^{-3} rad. or to much larger values as the position on the step, where the spacing of the fringe was measured,

approaches the centre of the crystal along the spiral step. The application of the same method to another specimen also gave results of the same order for the angle of rotational misorientation.

As for the small hole, we found it on some 60 of about 100 crystals with the spiral marking. Further, it can also be observed in Fig. 1*C* as marked by an arrow, where the original crystal has not suffered from any damage by the electron bombardment. Fig. 1*D* is an enlarged photograph of a hole which is a nearly regular hexagon in shape. Its edge line is oriented in parallel to each ridge line of the step. The growth spiral starts from one apex of the hexagon with round ridge line at the outset. Frank[7] has derived the relationship between the hole diameter (D_0) and the strength of the Burgers vector (b) at the core of the screw dislocation as follows, though slightly modified here:

$$b = 2\pi(S \cdot D_0/G)^{\frac{1}{2}} \qquad (1)$$

where S and G are the surface free energy and modulus of rigidity of the material. The hole diameter is distributed over a range of 200–2400 A. and the most probable value is concentrated in a range of 800–1200 A., while the mean value becomes 1020 A. With this value, the strength of the Burgers vector was estimated to be 93–118 A., where $2{\cdot}76 \times 10^{11}$ dyne/cm.[2] and 600–1,000 erg/cm.[2] were adopted for G and S[8] respectively. On the other hand, we have also estimated the step height to be 109 A. on an average by the shadow-casting method. This is of quite the same order as the strength of Burgers vector above obtained. Thus, if the step height is supposed to correspond directly to the Burgers vector, Frank's theory has been well proved in the present case.

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METALLURGY

Decomposition of the Beta-Phase in a Copper–Indium Eutectoid Alloy

Some optical and electron microscopical observations are reported of certain structures resulting from the decomposition of the beta-phase in a copper–indium alloy of approximately eutectoid composition.

The body-centred cubic beta-phase in the copper–indium system undergoes a eutectoid transformation, the eutectoid composition and temperature being approximately 31·4 wt. per cent indium and 574° C. respectively[1,2]. The product of this transformation is pearlitic, consisting of the alpha- and delta-phases. A detailed metallographic investigation of the isothermal transformation characteristics was made by Spencer and Mack[2]. Transformation at approximately 570° C. was found to occur by the formation of nodules of pearlite, resolvable under the optical microscope. The structure of nodules formed during transformation at 547° C. was not resolved optically, but it was reported that it was not homogeneous. Beta-phase, which had not transformed to pearlite, showed a rough-etching or cross-hatched structure when quenched to room temperature.

In the present work an alloy of approximately eutectoid composition was cast into a graphite mould of 3/8 in. diameter. The ingot produced was homogenized for three days at 650° C., and was then cut into disks of 3/32 in. thickness. Disks were quenched from 650° C. into a salt bath at either 565° C. or 547° C., and after being held for selected times at one of these temperatures, they were quenched into cold water. Disks were also quenched directly from 650° C. into cold water. Metallographic preparation of the heat-treated disks included mounting in 'Bakelite', grinding approximately 0·02 in. from the exposed 3/8-in. diameter surface, polishing with diamond dust and electrolytic etching with 1 per cent chromic acid. Examination was made by both optical and electron microscopy, the latter by means of unshadowed 'Formvar' replicas.

Optical microscopical examination (at × 1,400) of specimens partially transformed at 565° and 547° C. showed nodules which were partly resolvable as pearlite: electron microscopical examination of a number of replicas showed a completely pearlitic structure within the nodules. A portion of a nodule is shown in Fig. 1.

The beta-phase, which had not transformed to pearlite, when quenched, generally showed a rough etching appearance under the optical microscope, although there were variations in structure. Examination of replicas revealed the cellular structure illustrated in Fig. 1: a similar structure was shown by replicas taken from a specimen quenched directly from 650° C. into cold water. It may be concluded that it results from the decomposition

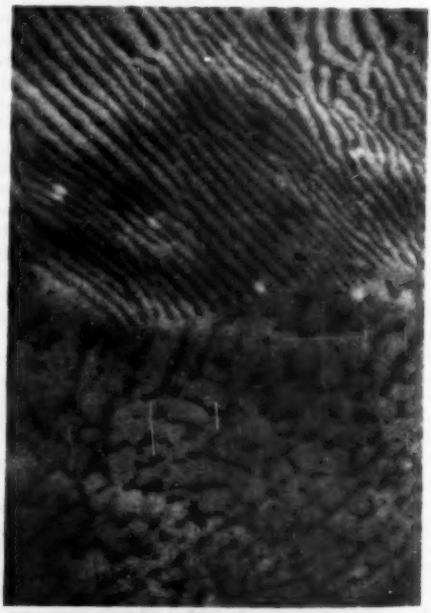

Fig. 1. Replica from specimen partially isothermally transformed at 547° C., showing a portion of a pearlite nodule with the adjacent region of quenched beta-phase (× about 25,000)

of the beta-phase during quenching. The cellular structure bears some resemblance to that observed under the optical microscope in copper–aluminium alloys which have been isothermally treated to produce a mixture of the disordered beta-phase and the ordered beta$_1$-phase (ref. 4 and Thomas, D. L., unpublished work). It is of interest to note that the diffraction pattern of the quenched copper–indium beta-phase has been found to contain lines which indicate a superlattice structure[1]. Thus, it seems possible that the cellular structure may be associated with the occurrence of an ordering reaction on quenching.

Optical microscopy showed that in some regions of the quenched beta-phase, immediately adjacent to the edge of the pearlite nodules, the structure was somewhat columnar in appearance. This effect occurred rather irregularly: where such columnar zones were observed they were commonly approximately 2μ in extent. A similar structural feature was reported by Spencer and Mack[3]. Examination of replicas confirmed that the cellular structure in these zones tended to adopt a columnar form. (This effect is not seen clearly in the particular micrograph shown.) The columnar zones may be associated with the presence of a strain gradient in the beta-phase adjacent to the pearlite nodule interface, the strain being due to volume changes accompanying pearlite formation. During quenching from the transformation temperature, the strain gradient might influence the morphology of the decomposition product of the beta-phase. In the examination of the replicas no interface was detected between the matrix of the cellular structure and one of the phases of the pearlite.

Acknowledgments are made to Mr. H. I. Matthews of the Department of Chemical Engineering, Imperial College, for the use of the Siemens Elmiskop I electron microscope in which the replicas were examined, to Dr. D. L. Thomas for helpful discussions, and to Miss P. Martins for assistance with the photographic work.

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Embrittlement of Tungsten and of Molybdenum

A recent paper from this laboratory[1] described the embrittlement of tungsten wire if it be heated in atmospheres containing even traces of ammonia. This work has been extended and has revealed a fact, unobserved in the original study, which furnishes further information which may be helpful in understanding the mechanism. In earlier experiments, the procedure was to heat a length of wire, leaving untreated ends which, since they had not been embrittled, could be clamped in the tensile testing machine without fear of fracture there. The disadvantage of this procedure is that the specimen under test had regions which reached lower temperatures than the central portion, so that any minimum in the embrittlement/treating-temperature curve would not be shown. This aspect was studied in the present series of tests. In these, wire was first annealed so that primary recrystallization was complete[2], and then reheated in nitrogen/hydrogen mixture containing 0·2 per cent ammonia. The whole specimens were then used for the test, neglecting those which broke in the clamps.

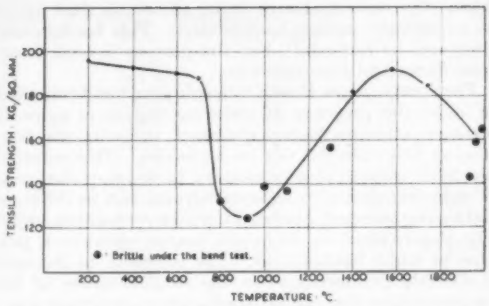

Fig. 1. Effect of heating for 10 min. in 0·2 per cent ammonia on the tensile strength at 20° C. of 180-μ tungsten wire

The results are given graphically in Fig. 1 and show that a minimum embrittlement does indeed occur after heating near 1,600° C.; above this, there is again further embrittlement. Both strength and bend tests were made; the latter were the more subjective but gave clear indications of qualitative changes: specimens which were brittle under this test are also indicated in Fig. 1.

Some recent work which we hope to publish soon has shown that embrittlement of molybdenum wires occurs on heating in presence of elementary nitrogen. While this effect can be important practically, it is quantitatively rather too small to reveal clearly any minimum in the mechanical properties. However, measurements of gas content of treated wires demonstrated that the absorption of nitrogen certainly shows a minimum near 1,300° C., which was not observed in the temperature-range studied by Tury[3], and it may be that there is a parallel mechanism operating for these two similar metals.

We thank Dr. J. A. M. von Moll and the directors of the Mullard Radio Valve Co., Ltd., for permission to publish this communication.

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CHEMISTRY

Benedict's Solution as a Developer in Carbohydrate Chromatography

While a large number of reagents have been developed for the differentiation of carbohydrates by chromatography, there is still some difficulty in showing reducing sugars in many biological materials. Many of the developers, such as silver nitrate, tend to become easily reduced and to stain the whole

paper, the carbohydrates being shown as dark spots on a similarly stained background. This background stain can be removed[1], but the process is both time-consuming and cumbersome.

For many years Benedict's solution has been used to show the presence of reducing sugars in urine, a positive reaction being obtained in solutions containing less than 0·1 per cent glucose. This solution has been used in this laboratory to develop the spots of reducing sugars in chromatograms run to differentiate galactose and glucose in urines containing sugar. The sugars show up as bright orange spots on a pale blue to white background. The intensity of the spot is directly proportional to the concentration of the carbohydrate, and can be used to give a rough estimate of concentration. Two solvents have been used to prepare the chromatograms. These are butanol/acetic acid/water and water-saturated phenol. This last is used in an atmosphere of ammonia. The solutions are allowed to run in descending chromatography for 24 hr.; 0·01 ml. of standard solutions of 1 per cent glucose and galactose are spotted on the base line. The urines are shaken for 20 min. with 'Amberlite *IR*-120' (H) resin and for a further 20 min. with 'Amberlite *IRA*-400' (OH) resin to remove cations and anions which cause tailing of the spots. 0·02 ml. of urine is spotted on the base line and the chromatogram is prepared as described above. The papers are removed from the developing tanks and dried at 80° C. and dipped into or sprayed with Benedict's solution. I find dipping more effective as it gives a more even distribution of the solution. The chromatograms are then developed by drying at 105° C. for 30 min. The spots are fairly permanent if the papers are kept in the dark after evaluation.

This technique has been used successfully for a wide variety of reducing sugars.

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A New Silicon Boride, SiB_4

The existence of compounds in the silicon–boron system has been a subject of conjecture for more than fifty years, since the many studies that have been made have produced negative, inconclusive, or contradicting results. Moissan and Stock[1], in the original work on the system, reported that by fusion of the elements they were able to prepare two compounds, SiB_3 and SiB_6. The SiB_3 was obtained as black rhombic plates with a density of 2·52 gm./cm.^{3}, and the SiB_6 as black irregular crystals with a density of 2·47 gm./cm.^{3}. The borides were reported to have high melting points, high hardness, and to be conductors of electricity, properties which prompted future workers to conduct further investigations.

Attempts by Tone[2] and Brewer[3] were unsuccessful. Studies of ternary systems involving silicon and boron[4] were uncertain regarding the silicon–boron binary, although a possible compound was indicated at about 90 per cent boron. Recently, Samsonov and Latysheva[5] have reported the properties of a tetragonal SiB_3 prepared by a method similar to that of Moissan. Their conclusions regarding the identification of the compound are doubtful, however, as Ormont *et al.*[6] have pointed out. Samsonov and Latysheva also prepared a compound SiB_6, which was studied by Zhuravlev[7], who reported a structure isomorphous with that of CaB_6.

Concurrent with the Russian work, Cline[8] prepared single crystals of SiB_6 and measured many of the physical properties[9]. The crystallographic data[10] indicate that the SiB_6 is orthorhombic and the measured density of 2·43 gm./cm.^{3} is much higher than the value of 2·15 gm./cm.^{3} reported by Zhuravlev for the cubic phase. Further experiments in the system involving the fusion of SiB_6 and silicon yielded a new phase which occurred as rhombs and hexagonal plates. The crystals are reddish-brown under reflected light and have a measured density (sink-float) of 2·44 gm./cm.^{3}. Chemical and spectrographic analyses indicate the formula SiB_4.

Rotation and Weissenberg zero- and upper-level photographs of single crystals of various forms of the SiB_4 indicate a rhombohedral lattice with $a = 5{\cdot}52$ A. and $\alpha = 69{\cdot}1°$. The corresponding hexagonal cell constants are $A = 6{\cdot}35$ A. and $C = 12{\cdot}69$ A. There are three molecules of SiB_4 in the rhombohedral unit cell. The probable space groups are $R32$, $R\bar{3}m$, and $R3m$. The density calculated from these data is 2·41 gm./cm.^{3}.

The physical properties of SiB_4 are under investigation, and these properties, as well as the details of the preparation, will be reported at a later date. Intensities are being measured, and a detailed examination of the structure will be made. SiB_4 appears to have some structural features in common with B_4C (ref. 11), and with various forms of elemental boron[12,13], and a comparison of the structures should prove extremely interesting.

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Products of Decomposition of Lead Azide

Previous work on the decomposition of alpha-lead azide have been performed at temperatures close to its ignition temperature[1,2]. The assumption has been made that the products of such decompositions have been only lead metal and nitrogen gas. The present communication describes the results of a study on the solid material obtained from the low-temperature thermal decomposition of alpha-lead azide.

The material used was a finely divided form of lead azide, prepared in as pure a form as possible. Crystal

Table 1. ALPHA-LEAD AZIDE HEATED AT 175° C. FOR 215 HR.

Copper $K\alpha$-radiation: product, $PbN_{2\cdot33}$ (γ-phase). $a_0 = 12{\cdot}25_4$A. Cubic indexed.

d obs.	Relative intensity	$\sin\theta$ (obs.)	$\sin^2\theta$ (obs.)	$\sin^2\theta$ (calc.)	$h^2+k^2+l^2$	h	k	l
8·67	5·4	0·0889	0·0079	0·0079	2	1	1	0
7·08	19·6	0·1089	0·0119	0·0119	3	1	1	1
4·09	10·9	0·1885	0·0355	0·0356	9	300	;	221
3·88	10·9	0·1987	0·0395	0·0396	10	3	1	0
3·06	100	0·2519	0·0635	0·0633	16	4	0	0
2·74	38·0	0·2814	0·0792	0·0792	20	4	2	0
2·67	22·8	0·2887	0·0834	0·0832	21	4	2	1
2·61	19·6	0·2954	0·0874	0·0871	22	3	3	2
2·40	5·4	0·3212	0·1032	0·1030	26	510	;	431
2·36	7·6	0·3267	0·1067	0·1069	27	511	;	333
2·28	5·4	0·3381	0·1143	0·1148	29	520	;	432
1·91	19·6	0·4036	0·1629	0·1624	41	621	;	540, 443
1·87	5·4	0·4122	0·1699	0·1702	43	5	3	3
1·67	10·9	0·4615	0·2131	0·2138	54	721	;	633, 552
1·61	10·9	0·4788	0·2293	0·2297	58	7	3	0
1·59	3·3	0·4848	0·2350	0·2335	59	731	;	553

Table 2. CHEMICAL ANALYSIS OF HEAT-TREATED ALPHA-LEAD AZIDE

Sample No.	Treatment	Nitrogen (per cent)	[Empirical formula	Phases	Colour
1	Untreated	28·90	$PbN_{6\cdot0}$	α-lead azide	white
2	Heated 150° C.	21·36	$PbN_{4\cdot25}$	phase 1 plus phase 2	light yellow
3	Heated 175° C.	17·80	$PbN_{3\cdot20}$	phase 2 plus phase 3	intense yellow
4	Heated 200° C.	16·86	$PbN_{2\cdot93}$	phase 2 plus phase 3	yellow-brown

Table 3

Compound	Lattice type and symmetry	Cell dimensions (A.)	Atoms per unit cell	Space group	Density (gm./cm.³)
Lead azide decomposition product, $PbN_{2\cdot5}$	Unknown deformed primitive; cubic	$a_0 = 12{\cdot}25_4$ $c_0 < a_0$	$Z = 25$		5·633
Metal, Pb	$A1$ cubic; face-centred	$a_0 = 4{\cdot}9396$	$Z = 4$	O^5_h; Fm^3m	11·341
PbO	$B10$ (tetragonal)	$a_0 = 3{\cdot}972$ $c_0 = 5{\cdot}023$	$Z = 2$	D^7_{4h}; $P4nmm$	9·35
Pb_3O_4	Unknown, tetragonal	$a_0 = 8{\cdot}80$ $c_0 = 6{\cdot}56$	$Z = 4$	D^{12}_{4h}; $P4/mbc$ or D^7_{2d}; $P\bar{4}b2$	8·95
$PbCO_3$	Unknown, orthorhombic	$a_0 = 5{\cdot}166$ $b_0 = 8{\cdot}468$ $c_0 = 6{\cdot}146$			

modifiers of all kinds were excluded. Cation impurities such as sodium, calcium, magnesium, iron, silicon and silver totalled less than 0·1 per cent. The mean particle size of the crystals was 7μ.

After thermal decomposition of the azide in fused silica crucibles, the solid residue was examined by X-ray crystallography using copper $K\alpha$-radiation. Both Debye–Scherrer and X-ray diffractograms were obtained. Samples weighing 0·250 gm. were decomposed under ambient pressure and temperatures of 150°, 175° and 200° C.

When the decomposition of lead azide is followed thermogravimetrically at a series of constant temperatures, weight-loss versus time curves like those in Fig. 1 are obtained. These results suggest that at least two phases in the decomposition of alpha-lead azide can be obtained, but the results do not exclude the possibility that the pauses in the curve may be the result of some unknown diffusion process. The results of a decomposition at 175° C., permitted to take place for a period of 230 hr., are shown in Fig. 2. The fact that diffusion is important in the process is evident from a comparison of the co-ordinates of the beginning of the first plateau in this figure and the same curve in Fig. 1.

In order to obtain more information on the nature of the pauses in the decomposition curve, decompositions at 175° C. were carried out for 215 hr. The result in Fig. 2 is representative. The constancy in the loss in weight at the first plateau is noteworthy.

The material which resulted from such decompositions had an intense yellow colour. X-ray diffractogram and Debye-Scherrer patterns were obtained on this material (see Table 1). The reflexions observed have been indexed as primitive cubic and they show excellent agreement between the observed and calculated $\sin^2\theta$ values. In addition, the calculation of the cell parameters suggests that the unit cell is not a perfect cube, since the c_0/a_0 ratio is not exactly 1.

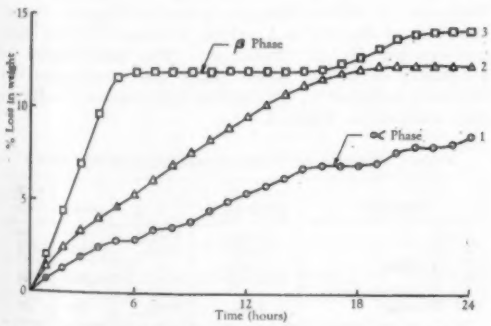

Fig. 1. Thermogravimetric study of decomposition of α-lead azide. (1) Decomposition at 150° C., α-phase; (2) decomposition at 175° C.; (3) decomposition at 200° C.

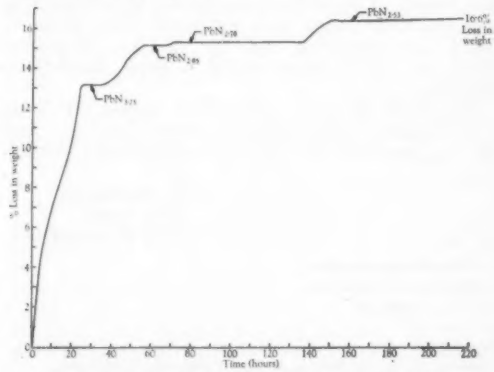

Fig. 2. Decomposition curve of α-lead azide at 175° C.

X-ray analyses of products of intermediate decompositions such as those characterized by the treatments in Fig. 1 suggest that a mixture of phases is present. Relative intensities are presented in Fig. 3 as functions of the reflexion angles. Nitrogen content by chemical analysis of these phases is shown in Table 2. Additional analyses for both lead and nitrogen were performed on a duplicate of sample 4 of this table. The lead was precipitated as lead sulphate and nitrogen determined by Dumas's method. The results in duplicate are :

Lead	Nitrogen
81·98 per cent	16·53 per cent
82·03	16·65
av. 82·00	av. 16·59

The material balance shows that oxygen can be present in the decomposition product only to the extent of 1·4 per cent if at all.

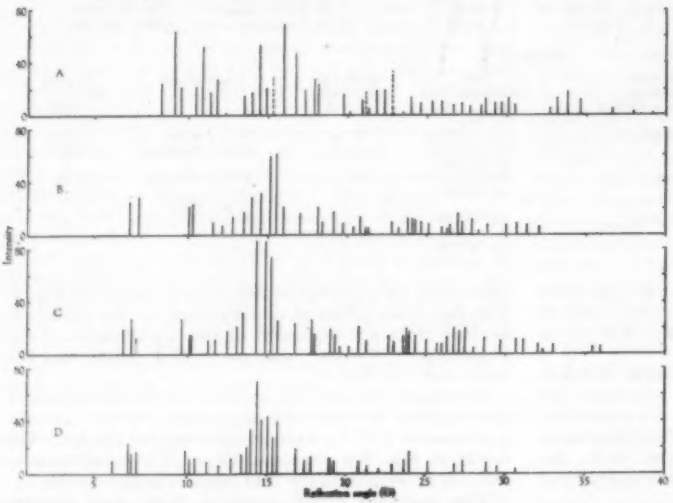

Fig. 3. Comparison of X-ray reflexions of untreated and heat-treated α-lead azide. (*A*) Untreated α-$Pb(N_3)_2$; (*B*) $PbN_{2\cdot92}$ α-$Pb(N_3)_2$ heated for 24 hr. at 150° C. ; (*C*) $PbN_{2\cdot80}$ α-$Pb(N_3)_2$ heated for 24 hr. at 175° C. ; (*D*) $PbN_{2\cdot09}$ α-$Pb(N_3)_2$ heated for 24 hr. at 200° C.

These results, plus an examination of data for probable materials which might result from heating alpha-lead azide in air as compiled in Table 3, suggests that under these conditions alpha-lead azide decomposes in a step-wise fashion through several distinct phases having varied lead to nitrogen ratios. The clarity of the diffractograms has been rewarding, and the very sharp lines exhibited have given no reason for assuming disorder in the structures. Work to confirm these results in more detail is in progress.

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Molecular Structure of Neopentane

We have recently studied the molecular structure of pivalonitrile[1], $(CH_3)_3CCN$, and have found the central C—C bond to be 1·46 ± 0·02 A. The carbon skeleton in pivalonitrile is similar to that in neopentane and, therefore, a comparison of the C—C distance in neopentane with the central C—C distance in pivalonitrile should be a measure of the effect of an adjacent triple bond on the C—C distance. It was also considered interesting to see if the C—C distance in neopentane would be longer than in ethane due possibly to steric repulsions. The only parameters available for neopentane were from the early visual work of Pauling and Brockway[2] and a re-investigation of the structure of this molecule seemed timely.

The sample used for the diffraction photographs was obtained from Phillips Petroleum Co. It was characterized as Phillips's research grade with a mole per cent purity of 99·92. Electron diffraction photographs were taken, using an r^3 sector, at camera distances of 25·10 cm. and 10·19 cm. employing electrons of wave-length 0·05452 A. and Kodak lantern slide plates. Exposure times of 10 sec. for the long camera distance and 30–45 sec. for the short camera distance were used. Three patterns at the short camera distance and two at the long camera distance were chosen for the visual measurement of ring diameters and estimation of intensities. The visual results from the sectored plates extended from $q = 17$ to $q = 100$.

A radial distribution curve was calculated from the sector-visual intensity data[3]. The data for the range $q = 0$ to $q = 17$ were supplied from a theoretical curve[3].

The radial distribution curve (Fig. 1) shows three peaks and a few shoulders. The peak at 1·09 A. corresponds to the bonded C—H distance and the one at 1·54 A. corresponds to the bonded C—C distance. The peak at 2·51 A. corresponds to the non-bonded C···C distance. The shoulder at 2·17 A. is attributed to the short non-bonded C···H distance. The shoulder at about 2·75 A. is due to the intermediate non-bonded C···H distance and the peak at 3·46 A. corresponds to the long non-bonded C···H distance. It is interesting to note that there is a small shoulder at about 1·8 A. which is caused by the short non-bonded H···H distance. The amplitudes of vibration of a few atom pairs were estimated by Karles's method[4,5]. The radial distribution results are summarized in Table 1.

Table 1. Results from the Radial Distribution Curve

Distance	Peak position (A.)	Δr_{ij}	Area[c]	$\frac{nZ \cdot Z_j}{r_{ij}}$
C—H	1·08$_5$	0·075	90	88
C—C	1·54$_4$	0·05	100	100
C···H (short)	2·16$_8$[a]	—		
C···C	2·51$_4$[b]	0·08	201	206
C···H (intermediate)	—	—		
C···H (long)	3·45$_5$	0·20$_4$	26	28

[a] This is consistent with a CCC angle of 109° 28′.
[b] Appears as a shoulder at about 2·75 A.
[c] Areas of the peaks were approximated to bell shapes.

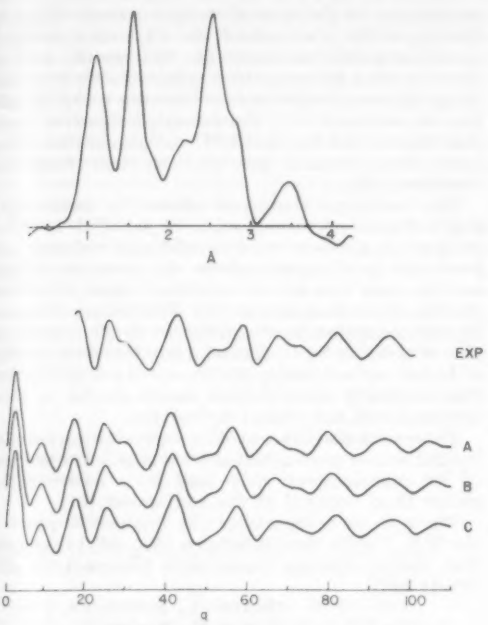

Fig. 1. The radial distribution curve and the experimental and theoretical intensity curves for neopentane

A correlation procedure has also been carried out. Six theoretical models with bonded C—C distances of 1·48 A. (*E*), 1·50 A. (*D*), 1·52 A. (*C*), 1·54 A. (*B*), 1·56 A. (*A*), and 1·58 A. (*AA*) were calculated. The bonded C—H distance and the CCC angle were maintained constant at 1·09 A. and 109·5° respectively. The hydrogen atoms were kept as far as possible from each other. Of these models all are qualitatively acceptable with the possible exception of the 1·48 A. model which is a borderline case. A model calculated with a bonded C—C distance of 1·54 A. assuming free rotation was found completely unacceptable. The quantitative electron diffraction results for the acceptable models are given in Table 2 and the parameters from the acceptable models are summarized in Table 3.

Table 2. Quantitative Electron Diffraction Results for Neopentane

Max.	Min.	q_{obs}	q_{calc}/q_{obs}					
			AA	*A*	*B*	*C*	*D*	*E*
3		17·50	0·985	0·996	1·005	1·018	1·037	1·051
	3	21·77	0·955	0·973	0·986	0·996	1·016	1·028
4		25·16	0·980	0·991	1·008	1·014	1·022	1·032
6		41·80	0·970	0·983	0·995	1·010	1·018	1·034
	6	47·24	0·984	0·999	1·007	1·022	1·030	1·044
8		57·99	0·957	0·969	0·979	0·991	1·002	1·011
	8	61·61	0·971	0·984	1·000	1·015	1·032	1·043
9		66·66	0·969	0·983	1·001	1·017	1·033	—
11		81·14	0·969	0·980	0·993	1·005	1·018	1·034
	11	87·08	0·981	0·991	1·001	1·011	1·023	1·035
12		93·83	0·987	0·997	1·009	1·021	1·033	1·044
	12	99·62	0·988	0·999	1·012	1·023	1·035	1·050
Mean q_{calc}/q_{obs}			0·975	0·987	1·000	1·012	1·025	1·037
Average deviation from the mean			0·010	0·008	0·008	0·008	0·008	0·009

Table 3. Parameters from Acceptable Models

	AA	*A*	*B*	*C*	*D*	*E*
C—H	1·06$_5$	1·07$_5$	1·09$_0$	1·10$_5$	1·11$_7$	1·13$_0$
C—C	1·54$_1$	1·54$_0$	1·54$_0$	1·53$_8$	1·53$_8$	1·53$_5$

The final parameters of the molecule from this study are:

$$C—C = 1·54 \pm 0·01 \text{ A.}$$
$$\angle CCC = 109·5 \pm 1°$$

No attempt was made to determine the C—H distance as any such determination would result in an uncertainty of at least ±0·05 A.

It is interesting to note that the C—C distance in neopentane is equal to the value of 1·54 A., which has usually been accepted as the normal C—C single-bond distance. Within the estimated uncertainty, this distance is also consistent with the value of 1·533 A. proposed by Bartell[6] as the normal C—C single bond distance in straight-chain hydrocarbons. The interatomic distances obtained in this investigation are in agreement with those obtained earlier by Pauling and Brockway[2], but the present work is regarded as more accurate.

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Crystallization of Non-Racemic Mixtures of the Isomers of Serine

If the enantiomorph (unlabelled) of a labelled isomer is available, the maximum contamination of the particular labelled compound by its enantiomorph may be assessed by a crystallization method. Repeated crystallization of a mixture of enantiomorphs in which one (unlabelled) is present in large excess of the other (labelled) would be expected to lead to a purification of the more abundant isomer; hence, the presence of isotope in the purified abundant isomer should represent contamination of the labelled antipode. Thus, when enzymatically prepared[1,2] D-glutamic acid-2-^{14}C was diluted about 2,000-fold with carrier L-glutamic acid and the mixture crystallized, via the hydrochloride and the free amino-acid, to constant specific activity, it could be calculated that the maximum amount of contaminant L-glutamic acid-2-^{14}C was less than 1 per cent[3].

We have resolved DL-serine-3-^{14}C (obtained from Nuclear-Chicago Corp.) by the selective enzymatic hydrolysis of the N-chloroacetyl derivative[2]. To estimate the purity of the labelled D- and L-serine samples, the latter were diluted about 2,000-fold with carrier D- or L-serine and subjected to repeated recrystallization from water or ethanol–water mixtures. Aliquots of the various crystalline products obtained were assayed for carbon-14[4]. The results are presented in Table 1. Surprisingly, when the labelled serine isomers were diluted with their respective antipodes (^{14}L + D, or ^{14}D + L), successive

Table 1. Specific Activities after Successive Recrystallizations of D- or L-Serine-3-^{14}C Diluted about 2,000-fold with Carrier D- or L-Serine*

No. of recrystallization	Range of yield	μc./m.mole† ^{14}L+D	^{14}L+L	^{14}D+L	^{14}D+D
A Serine (first series)					
4	40–52‡	913	492	898	494
5	76–90§	1,122	485	1,139	494
6	64–76§	1,545	462	1,650	478
7	20–24§	5,200	280	5,740	331
B Serine (second series)					
Dried aliquot	—	470	487	493	483
1	93–96†	495	485	509	476
2	12–18§	1,710	368	3,390	387
3	30–45§	—	—	5,900	—
C *p*-Toluenesulphonyl derivative of product of first crystallization in *B* (above)					
2		22	503	22	467
3		3·8	—	8·6	—
4		—	—	5·9	—

* Obtained commercially: $[\alpha]_D^{27}$ $-11{\cdot}8 \pm 1°$ (D-serine).

$[\alpha]_D^{27}$ $+12{\cdot}8 \pm 1°$ (L-serine)

† Corrected for dilution with carrier. Therefore, the results give the relative specific activities of each precipitate.
‡ Per cent (by weight) of original mixture recovered.
§ Per cent (by weight) of previous crystallization recovered.

recrystallization led to an increase in specific activity, the increase being approximately inversely proportional to the recovery (by weight) during a given crystallization. Thus, in the case of ^{14}D + L (Series *A*, seventh recrystallization), 4·2 per cent of the original serine contained 49 per cent of the original carbon-14, an enrichment of isotope of more than eleven-fold. Repeated recrystallization of ^{14}L + L or of ^{14}D + D gave products of fairly constant specific activity except when the recovery of serine was low, in which case the specific activities fell markedly.

These unusual results can be explained only by assuming that the carrier D- and L-serine used were contaminated with several per cent of their respective enantiomorphs, and that during recrystallization the contaminants were preferentially precipitated. Apparently, the DL-component of each mixture was almost quantitatively precipitated under conditions in which the L- or D-isomer was quite soluble. The lesser solubility of DL-serine as compared with D- or L-serine[5] is in accord with this hypothesis, although the difference in solubilities does not afford a complete explanation of the results.

Optical rotations, determined spectrophotometrically, support the above hypothesis. (Optical rotations were determined by using a Keston polarimeter attachment to a Beckman Model *DU* spectrophotometer (Standard Polarimeter Co., Hackensack, New Jersey, U.S.A.). All rotation measurements were made at 589 mμ using 5 *N* hydrochloric acid as solvent (temperature 24–27° C.)). The specific rotations of the carrier D- and L-serine were, respectively, $-11{\cdot}8 \pm 1°$ and $+12{\cdot}8 \pm 1°$. Specific rotations ranging from 0 to $4{\cdot}60 \pm 3°$ were obtained on the final precipitates of the two serine recrystallization series (Table 1). Greenstein *et al.*[3] have reported specific rotations for pure serine isomers (in 5 *N* hydrochloric acid) of about 15°.

In contrast to the results with serine, recrystallization of the *p*-toluenesulphonyl derivatives gave a purification of the more abundant isomer. Thus, in the case of ^{14}D + L (series *B* and *C*), recrystallization as serine yielded a precipitate the specific activity of which was 1,000 times that obtained after recrystallizing the *p*-tosyl derivative of the same serine sample. Results obtained with the *p*-tosyl derivatives prove that the D- and L-serine-3-^{14}C samples contained no more than about 1 per cent of their respective enantiomorphs.

The technique described above for assessing a slight degree of racemization in labelled D- or L-compounds appears to be a valid and valuable one. However, in instances where the racemate is less soluble than the D- or L-isomers, large errors are possible if the isomers used for dilution are not pure. In this connexion we found that a single crystallization of D-serine-3-^{14}C, diluted 5,000-fold with L-serine of higher optical purity ($[\alpha]_D^{27} = +14{\cdot}6 \pm 1°$) than that originally used, yielded results similar to those obtained with the *p*-tosyl derivatives.

The results also illustrate that recrystallization of an optical isomer contaminated with only a few per cent of its enantiomorph may lead to a concentration rather than removal of the contaminant.

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A New Route to 1-Substituted Pyrazole-4-Carboxylic Acids

1-Phenylpyrazole-4-carboxylic acid has been hitherto most successfully synthesized by chloromethylation of 1-phenylpyrazole and subsequent oxidation of the 4-chloromethyl-1-phenylpyrazole[1], or by hydrolysis of the 4-cyano derivative formed from the anti-oxime of 4-formyl-1-phenylpyrazole[2]. A synthesis of this acid has now been achieved by the hydrolysis of 4-cyano-1-phenylpyrazole formed by a Sandmeyer type reaction from 1-phenylpyrazole-4-diazonium chloride. Replacement of the pyrazole-4-diazonium group by nitrile has hitherto been unsuccessful[3,4].

The starting material, 4-nitro-1-phenylpyrazole, was prepared in quantitative yield by the method of Hill and Torrey[4]. Reduction by hydrazine catalysed by Raney nickel[5] yielded 4-amino-1-phenylpyrazole (97 per cent). This reagent we found to be an excellent reductant for 4-nitropyrazoles. The following pyrazoles: 4-nitro-1-2′-quinolyl-, 1-2′-benzthiazolyl-4-nitro-, and 4-nitro-1-2-5-trimethyl- have also been reduced to the corresponding amines in yields varying between 87 and 100 per cent (unpublished work).

4-Amino-1-phenylpyrazole was diazotized and added to a potassium cuprocyanide solution at 80°.

Ether extraction gave a 56 per cent yield of 4-cyano-1-phenylpyrazole, m.p. 94–95° (Lit.[3] 95°). Found: C, 70·8; H, 4·4; N, 24·8. Calc. for $C_{10}H_7N_3$: C, 71·1; H, 4·1; N, 24·8 per cent. A quantitative yield of 1-phenylpyrazole-4-carboxylic acid, m.p. 221–222°, was obtained on hydrochloric acid hydrolysis of the 4-cyanopyrazole.

The overall yield of 1-phenylpyrazole-4-carboxylic acid by this synthesis is greater than 50 per cent and the route should provide easy access to other 1-substituted pyrazole-4-carboxylic acids.

There is but one reference in the literature to a similar substitution by this method of a pyrazole-4-diazonium group by halogen and the yields were poor[6]. We have found that, utilizing cupric salts as catalysts at elevated temperatures, halogen can be introduced in good yield, for example, 1-phenylpyrazole-4-diazonium bromide gives 4-bromo-1-phenylpyrazole in 86 per cent yield.

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BIOCHEMISTRY

3 : 3′-Diamidinocarbanilide: a New Drug Active against Babesial Infections

Quinuronium sulphate[1] is the commonly employed treatment for British bovine redwater and a single injection of 1 mgm. of this drug per kgm. of body-weight is effective in a high proportion of cases. But quinuronium sulphate has the disadvantage that even in therapeutic doses it produces toxic effects associated with parasympathetic stimulation. The diamidines, phenamidine and pentamidine, as their di-isethionates, and Berenil are well tolerated and are also used for the treatment of bovine babesiasis, but they are less active than quinuronium sulphate.

During the search for a new drug which would be at least as effective as quinuronium sulphate but without its toxic effects, a large number of compounds has been screened for activity against *Babesia rodhaini* in mice, and the most promising of these were tested against *Babesia divergens* in splenectomized calves. One of the best of these was M and B 5062*A* — 3 : 3′ - diamidinocarbanilide di-isethionate, which was active against *B. divergens* in calves and produced none of the toxic effects associated with quinuronium sulphate. This activity of the 3 : 3′-diamidine was unexpected because of the inactivity of the corresponding 4 : 4′-diamidine. 3 : 3′-Diamidinocarbanilide di-isethionate[2] was prepared, among other methods, by the usual Pinner reaction on the corresponding dinitrile. It is a white, crystalline, anhydrous solid, m.p. 209° C. (decomposing at 256° C.) (found: N, 15·1; S, 11·6 per cent. $C_{18}H_{18}ON_6,2C_2H_6O_4S$ requires: N, 15·3; S, 11·7 per cent), and is approximately 100 per cent w/v soluble in water.

The acute toxicity (*LD*50) of M. and B. 5062*A* in mice was found to be 120 mgm./kgm. subcutaneously compared with 8 mgm./kgm. for quinuronium sulphate, while a comparison of the two compounds in non-infected, splenectomized calves after subcutaneous injection at a site behind the shoulder showed that the maximum tolerated dose of M. and B. 5062*A* was greater than 40 mgm./kgm., and that of quinuronium sulphate less than 4 mgm./kgm. There were no obvious symptoms of toxicity in the calves that received this dose of M. and B. 5062*A*. During the therapeutic activity tests two calves developed mild ataxia which lasted for about 3 hr. after treatment with a dose of 40 mgm./kgm.; otherwise no symptoms of systemic toxicity were observed.

In the therapeutic activity tests, seventeen calves infected, after splenectomy, with *B. divergens* were treated subcutaneously with M. and B. 5062*A* at doses between 3 and 40 mgm./kgm. body-weight. With all doses the intensity of the parasitæmia was reduced within 24 hr. and complete clearance was obtained within three days. In all the calves treated with 5 mgm./kgm. or higher doses, the hæmoglobinuria was cleared in 24 hr.

Further details, including a report on field investigations, will be published in due course.

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Metabolism of l-^{14}C Lignoceric Acid in the Rat

Lignoceric acid, the straight-chain, saturated C_{24} fatty acid, occurs in sphingolipids in amide linkage with the amino-alcohol sphingosine[1,2]. It has not been found in the ester linkages of neutral fat or glycerophosphatides, and very little information is available on its metabolism. In the work reported here, lignoceric acid labelled with ^{14}C carboxyl was administered to rats and its metabolic fate studied.

Lignoceric acid was isolated from peanut oil and converted to 1-^{14}C lignoceric acid, via 1-^{14}C tricosyl cyanide (modification of Anker's method for synthesis of myristic acid[3]). Contrary to the fatty acids of 18 carbon atoms or less, lignoceric acid did not form a soluble complex with serum albumin. Paper electrophoresis of a mixture of 1-^{14}C lignoceric acid and serum albumin (0·5–1 equivalent of the acid per equivalent of albumin) showed that the radioactive fatty acid did not migrate with the albumin but remained at the point of origin. 1-^{14}C lignoceric acid was therefore injected as a neutralized aqueous suspension into the tail vein of rats; the various organs were isolated and extracted with a 2:1 chloroform methanol mixture. Respiratory carbon dioxide labelled with carbon-14 was collected in some experiments, and, in others, the livers were removed, dried and extracted successively with acetone, petroleum ether and ethanol. The acetone and petroleum

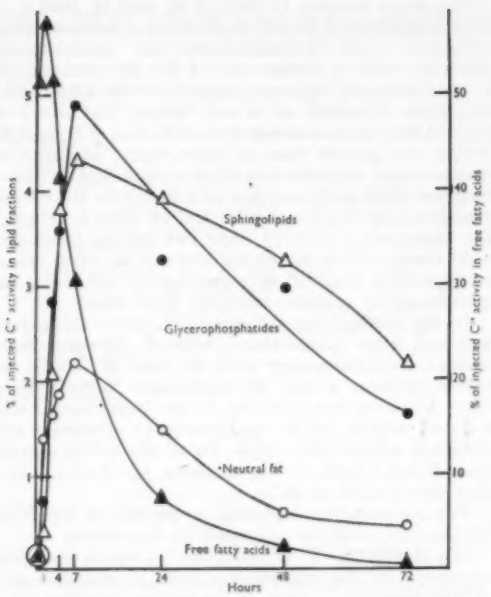

Fig. 1. Incorporation of 1-^{14}C lignoceric acid into rat liver lipids. 6 μmoles of 1-^{14}C lignoceric acid (94,000 c.p.m.) were given by intravenous injection to rats. At the specified times, the animals were killed and the liver lipids fractionated as described in the text

ether extracts were combined and the lipids separated into a petroleum ether and acetone soluble fraction (that is, neutral fat and free fatty acids) and a petroleum ether soluble, acetone insoluble fraction (that is, glycerophosphatides)[4]. Sphingolipids were isolated from the ethanol extract. The neutral fat was separated from the free fatty acids by passing through a magnesia-'Celite' column[4]. The glycerophosphatides were purified by repeated precipitations of their petroleum ether solutions with acetone and the sphingolipids by repeated precipitations of their ethanol solutions with acetone.

Within 15 min. after injection, about 99 per cent of the 1-^{14}C lignoceric acid was removed from the blood. More than 50 per cent of the injected radioactivity was found in the liver. Small amounts were present in the lungs and spleen; no radioactivity was found in the brain and only traces in the adipose tissue. A similar pattern of distribution was observed after 24 hr.

About 20 per cent of the radioactivity of the administered 1-^{14}C lignoceric acid appeared in the respiratory labelled carbon dioxide in 7 hr. After an initial lag of about 30 min., the rate of production of labelled carbon dioxide increased, reached a maximum 2 hr. after injection and then declined slowly.

Contrary to the findings with the lower fatty acids[5,6], feeding a stock diet, a diet rich in carbohydrates or fasting, did not influence the formation of labelled carbon dioxide from 1-^{14}C lignoceric acid.

Both the rate of incorporation of 1-^{14}C lignoceric acid into liver lipids and the turnover-rate of these lipids (Fig. 1) were much lower than with palmitic acid[7]. Also, contrary to palmitic acid[7], a large fraction (about 50 per cent) of the injected lignoceric acid was found as the free acid in the liver; even after 24 hr. about 8 per cent of the free fatty acid was still present in the liver.

A considerable part of the lignoceric acid incorporated into liver lipids was found in the sphingolipids (Fig. 1). However, 1-^{14}C lignoceric acid was also found in the neutral fat and glycerophosphatides. This esterification of lignoceric acid, which has not been observed previously, might be due to the presence, in unphysiological amounts, of free lignoceric acid in the liver.

The results presented indicate that while 1-^{14}C lignoceric acid was metabolized by the rat, its metabolic behaviour differed from that of lower fatty acids, such as palmitic acid. Experiments with tissue preparations are in progress to elucidate further the above findings.

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Ribonucleases in Takadiastase

The existence of a thermostable ribonuclease in commercial takadiastase was first pointed by Kuninaka[1]. We found afterwards by zone-electrophoresis the existence of at least three enzymes attacking ribonucleic acid in takadiastase: one of them is more active at *p*H 7·5 and the others are more active at *p*H 4·5 [2].

Two of the enzymes, which we named ribonuclease T_1 and ribonuclease T_2, were partially purified by the following procedure: heat treatment, ammonium sulphate fractionation, adsorption to and elution from calcium phosphate gel. Ribonuclease T_1 was further purified by eliminating impurities with active charcoal, and T_2 by chromatography with *DEAE*-cellulose[2,3]. The preparations thus obtained of ribonuclease T_1 and T_2 were shown to be practically homogeneous in zone-electrophoresis.

Ribonuclease T_1 split the internucleotide bonds between 3′-guanylic acid groups and 5′-hydroxyl groups of the adjacent nucleotides with the intermediary formation of guanosine-2′,3′-cyclic phosphate[2–5].

This mechanism was deduced from the following experimental evidence: (1) ribonuclease T_1 digesting yeast ribonucleic acid produces exclusively 3′-guanylic acid (*Gp*) as mononucleotide; (2) the terminal residue of oligonucleotides produced was shown to be exclusively guanylic acid; (3) as an intermediate of the reaction, guanosine-2′,3′-cyclic phosphate ($G_{\text{cyclic P}}$) was obtained with good yield, and it was further hydrolysed to 3′-guanylic acid:

$$\begin{array}{c} \text{—}XpGpXp\text{—}XpGpGpXp\text{—} \\ \downarrow \text{RNase } T_1 \\ \text{—}XpG_{\text{cyclic P}} + Xp\text{—}XpG_{\text{cyclic P}} + G_{\text{cyclic P}} + Xp\text{—} \\ \downarrow \text{RNase } T_1 \\ \text{—}XpGp + Xp\text{—}XpGp + Gp + Xp\text{—} \end{array}$$

G, guanosine
X, other nucleosides

Similar experiments were undertaken with ribonuclease T_2, and in this case it was proved that it splits the internucleotide bonds between 3′-adenylic acid groups and 5′-hydroxyl groups of the adjacent nucleotides. However, contrary to the reaction catalysed by ribonuclease T_1, cyclic mononucleotides were not readily obtained[3].

The specificity of ribonuclease T_1 and T_2 is indeed just complementary to that of the well-known pancreatic ribonuclease (ribonuclease 1), at least the main component, component *A*, which catalyses the cleavage of internucleotide bonds between the 3′-pyrimidine nucleotide groups and the 5′-hydroxyl groups of adjacent nucleotides[6].

Some properties of ribonuclease T_1 and T_2 together with ribonuclease 1 are summarized in Table 1.

Table 1. PROPERTIES OF RIBONUCLEASE T_1, T_2 AND *I*

	Optimum *p*H	Specificity	Activation and inhibition				References
			Mg^{++} Ca^{++}	Cu^{++}	EDTA	ICH_2COOH	
T_1	7·5	*Gp*	inhibited	inhibited	activated	activated	(2) (4) (5)
T_2	4·5	*Ap*	inhibited	strongly inhibited	activated	activated	(2) (3)
I	7·6	*Up*	activated	inhibited		inhibited	(6)
		Cp					

Ribonuclease T_1 and T_2 catalyse the synthesis of oligonucleotides from guanosine-2′,3′-cyclic phosphate ($G_{\text{cyclic P}}$)[7,8] and adenosine-2′,3′-cyclic phosphate ($A_{\text{cyclic P}}$)[8] respectively. For example, ribonuclease T_1 catalyses the synthesis of guanylyluridine, guanylylcytidine, guanylyladenosine and guanylylguanosine from cyclic guanylic acid and uridine, cytidine, adenosine and guanosine respectively. It catalyses also the polymerization of cyclic guanylic acids. Part of the products are poly- or relatively large oligo-guanylic acids, because they are non-dialysable and give the colour reaction by gallocyanine[9].

$$G_{\text{cyclic P}} + U = GpU \qquad G_{\text{cyclic P}} + C = GpC$$
$$G_{\text{cyclic P}} + A = GpA \qquad G_{\text{cyclic P}} + G = GpG$$
$$nG_{\text{cyclic P}} = GpGpGp\text{—}Gp.$$

Similar reactions were also observed with ribonuclease T_2. These synthetic reactions are analogous to those of oligopyrimidine nucleotides catalysed by ribonuclease I[10]. The generality of such synthetic reactions by certain ribonucleases suggests the possibility of the participation of these enzymes in the intracellular synthesis or reformation of specific ribonucleic acids.

Preparation of oligo- and poly-guanylic acids and cyclic nucleotides by ribonuclease T_1. As mentioned above, ribonuclease T_1 catalyses the polymerization of cyclic guanylic acid. By fractionation on *ECTEOLA*-cellulose column, oligo- and poly-guanylic acids were obtained[8].

K. Sato-Asano and Y. Fujii showed that deaminated yeast ribonucleic acid could be hydrolysed by ribonuclease T_1[11]. By controlled digestion of yeast ribonucleic acid and deaminated yeast ribonucleic acid, guanosine-2′,3′-cyclic phosphate and two new compounds, inosine-2′,3′-cyclic phosphate and xanthosine-2′,3′-cyclic phosphate, were obtained respectively with good yields[12].

Abolition of the streptolysin-forming activity of ribonucleic acid. An interesting biological effect of ribonucleic acid was disclosed by Okamoto's finding that yeast ribonucleic acid stimulated the synthesis of streptolysin ('Streptolysin *S*') by hæmolytic streptococci[13]. The streptolysin-forming activity of ribonucleic acid was rapidly decreased by ribonuclease T_1[14]. This finding is consistent with the observations which suggest that the activity depends on the consecutive sequence of guanylic acids[15].

Possibility of the determination of nucleotide distribution in ribonucleic acid. As various proteinases have contributed to the determination of amino-acids sequence in protein, ribonucleases with different specificity may be useful for the elucidation of the structure of ribonucleic acid.

In Table 2 the hydrolysis of a hypothetical polynucleotide by ribonuclease *I*, T_1 and T_2 is presented. By combining the information about the nucleotide sequences of hydrolysis products, the complete nucleotide sequence may be deduced[8].

Table 2. HYDROLYSIS OF A HYPOTHETICAL POLYNUCLEOTIDE

	G-A-U-C-C-G-A-A-G-G-C-U-U-G-A-C-U
Ribonuclease *I*	*G-A-U C C G-A-A-G-G-C U U G-A-C U*
Ribonuclease T_1	*G A-U-C-C-G A-A-G G C-U-U-G A-C-U*
Ribonuclease T_2	*G-A U-C-C-G-A A G-G-C-U-U-G-A C-U*

Native ribonucleic acids so far obtained are too large and may be a mixture of polynucleotides with different nucleotide sequences. However, by analysing mono- and oligo-nucleotides produced by the three ribonucleases and applying simple probability calculation, we may be able to determine how the nucleotide sequence deviates from that of random distribution. The information thus obtained may be an indication for the specificity of nucleotide distribution of the ribonucleic acid.

In conclusion, we believe that ribonuclease T_1 and T_2 will prove useful for studies of nucleic acids and related problems.

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Occurrence of Riboflavin in Sturgeon Skin

LOOMEIFER[1] has reported the presence of a yellow fluorescent pigment in elastin and has commented upon the views of Szent-Györgyi[2] on the function of riboflavin-5-phosphate in metabolism as an energy transmitter. Blair and Graham[3] found riboflavin to be present in the skin of green snakes. These reports of fluorescent materials associated with connective tissues, which are metabolically relatively inert,

have prompted this note of the occurrence of riboflavin in sturgeon skin. These observations form part of a survey on non-teleostean fish collagen.

The skin of a young (7 kgm.) sturgeon (*Acipenser sturio*) was mechanically freed from muscle and scales and heated in water, adjusted with acetic acid to *p*H 4·5, for 3 hr. at 60° C. in the dark. Approximately 80 per cent of the collagen was converted into gelatin, which, as a 5·6 per cent (w/v) solution, gave the physical properties shown in Table 1, and compared with gelatins from isinglass (*Acipenser huso huso*), shark skin (*Selachus maximus*) and cod swim-bladder (*Gadus morrhua*) which were prepared under similar conditions.

Table 1. Properties of Fish Gelatins

	gelatin 5·6% (w/v)	
	rigidity dynes/cm.[2] at 10° C.	viscosity cp. at 40° C.
Sturgeon skin	25,000	5·2
Isinglass	60,000	5·8
Shark skin	45,000	4·3
Cod swim-bladder	*c.* 100	4·0

The gelatin solution from the sturgeon skin was unusual in that it was associated with a yellow pigment showing a strong green fluorescence. The fluorescence was destroyed by sunlight. The absorption spectrum of this gelatin, plotted between 380 and 580 mμ, was found to be similar (Fig. 1) to that for an acid-processed pig skin gelatin to which riboflavin had been added. In addition, when the sturgeon skin gelatin was irradiated with an ultra-violet source for 30 min. and the absorption curve plotted, it was found that the hump shown by curve *D*, Fig. 1, had been removed so as to resemble curve *B*. The plot of the differences in absorption between the irradiated and non-irradiated forms is shown in Fig. 2. The maximum at approximately 455 mμ corresponds to the maximum given by riboflavin. It seems likely, therefore, that riboflavin is the colour component of this sturgeon gelatin.

It remains to be seen whether the presence of riboflavin in sturgeon skin is a pigmentation phenomenon or whether it is a metabolic factor involved in the laying down of collagen in a young animal.

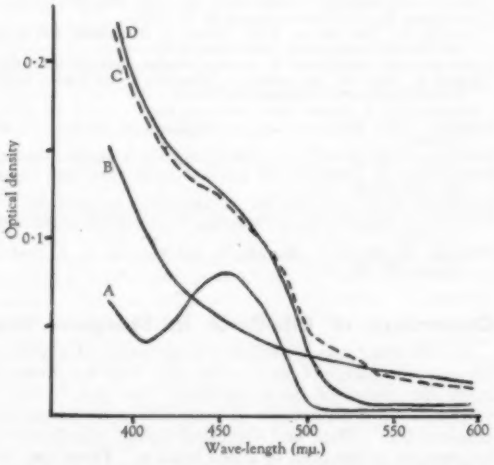

Fig. 1. Absorption spectra: curve *A*, riboflavin; curve *B*, pig skin gelatin; curve *C*, pig skin gelatin with added riboflavin; curve *D*, sturgeon skin gelatin

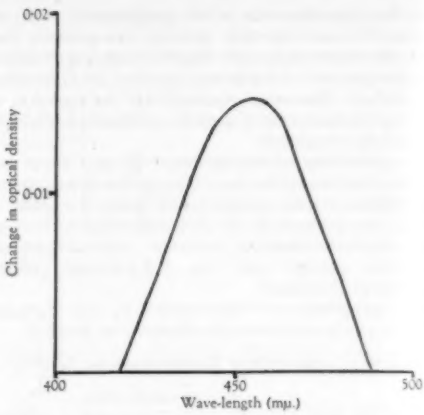

Fig. 2. Changes in the optical densities of sturgeon skin gelatin occurring after ultra-violet irradiation

I am most grateful to H.M. The Queen and to the Comptroller of Supplies, Buckingham Palace, for arranging the gift of a sturgeon.

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Properties and Solubility of Phenolase in Isolated Chloroplasts

The phenolase complex in green plant tissue has often been assumed to be present only in the chloroplasts[1]; but it has recently been shown that in sugar beet leaf phenolase is also present in the mitochondria and 'soluble' fraction of homogenates. The enzyme associated with chloroplasts was shown to be characterized by a high cresolase activity, the ratio of catecholase to cresolase activities being close to unity. Disruption of such chloroplasts with distilled water increases both activities by 35 per cent[2].

In order to examine further the chloroplast phenolase of sugar beet leaf, we have attempted to extract it by a number of methods. The preparation of chloroplasts and manometric determination of enzymic activities have been described previously[2]. Chloroplasts were macerated by hand in an all-glass homogenizer with the solutions described in Table 1, left to stand for the time indicated and then separated by centrifugation for 5 min. at 3,700*g*. All the preparations were carried out in a cold room at − 1° C.; the acetone had previously been cooled to − 20° C.

The treatments with either sodium deoxycholate or acetone (Table 1, *a*–*e*) increased the total amount of phenolase activity compared with that in whole chloroplasts. The increases ranged from two and a half-fold with acetone (*c*) to six-fold with acetone and sodium deoxycholate (*e*). With the exception of treatment (*a*), all the extracts (*b*–*e*) had increased catecholase/cresolase ratios when compared with the original whole chloroplasts. The first treatment (*a*)

Table 1. Specific Activities, Total Activities, Distribution of Activity and Increase of Activity resulting from Extraction of Isolated Chloroplasts by Various Means. Specific Activity is Maximal Rate of Oxidation of Substrate measured as μL. Oxygen/mgm. Protein/min.

Experiment	Extraction treatment*	Fraction	Specific activity: Catecholase	Specific activity: Cresolase	Ratio Cat./Cres.	Total activity (catecholase) in fraction (specific activity × mgm. protein in fraction)	Percentage of total activity extracted into solution†	Increase in activity‡
a	1 per cent sodium deoxycholate, *p*H 7·3§, 30 min.	1 whole chloroplasts	1·13	1·13	1·0	34		
		2 extract	4·9	4·9	1·0	108	70	× 4·5
		3 residue	6·0	5·5	1·09	46		
b	1 per cent sodium deoxycholate, *p*H 6·3§, 10 min.; residue then treated with 1 per cent sodium deoxycholate, *p*H 7·3§ for 10 min.	1 whole chloroplasts	1·17	1·17	1·0	43		
		1a *p*H 6·3 extract	0	0		0		
		2 *p*H 7·3 extract	7·5	5·6	1·43	114	31	× 4·0 (allowing for totally inactive protein fraction 1a)
		3 *p*H 7·3 residue	9·2	6·7	1·36	250		
c	Acetone¶ followed by buffer *p*H 7·3, 10 min.	1 whole chloroplasts	2·25	1·9	1·17	53		
		2 extract	7·4	5·2	1·42	70	52	× 2·5
		3 residue	3·5	2·6	1·32	64		
d	Acetone¶ followed by buffer *p*H 8·0, 10 min.	1 whole chloroplasts	2·25	1·9	1·17	53		
		2 extract	12·5	6·0	2·1	52	39	× 2·5
		3 residue	4·65	3·9	1·17	81		
e	Acetone¶ followed by 1 per cent sodium deoxycholate, *p*H 7·3§, 10 min.	1 whole chloroplasts	2·25	1·9	1·17	53		
		2 extract	29·2	17·0	1·7	162	51	× 6·0
		3 residue	8·5	6·45	1·32	155		
f	1 per cent digitonin, *p*H 7·3§, 10 min.	1 whole chloroplasts	2·0	1·74	1·17	43		
		2 extract	3·9	1·8	2·6	26·5	67	× 1·0 (that is, no activation)
		3 residue	2·08	2·47	0·84	13·0		

* This shows the solution in which the chloroplasts were macerated, and the time during which they were left at 0° C. before centrifuging.

† $\frac{\text{Total activity in extract}}{\text{Total activity in extract + T.A. in residue}} \times 100$

‡ $\frac{\text{Total activity in extract + T.A. in residue}}{\text{Original total activity of whole chloroplasts}}$

§ Both the sodium deoxycholate and digitonin were dissolved in *M*/15 phosphate buffer at the *p*H's indicated above.

¶ Chloroplasts rapidly macerated with acetone and then centrifuged. Total time of contact therefore 5½ min. Acetone residue then extracted as indicated above.

yielded the highest amount of activity in the extract (~70 per cent).

Digitonin (Table 1) also extracted about 70 per cent of the total phenolase, but in this case the extract had a greatly increased catecholase/cresolase ratio, and the residue a decreased one; furthermore, there was no increase in activity after the treatment. The activation of chloroplast phenolase by acetone and deoxycholate, on one hand, and the lack of activation by digitonin, on the other, may be comparable phenomena to those observed by Kenten[3] for the latent phenolase of bean leaf. He found that the latent phenolase is activated by anionic but not by non-ionic wetting agents.

It is important to note that with the treatments used, not all the phenolase could be extracted from the chloroplasts. This finding suggests that the enzyme is either tightly bound to, or forms part of, some structure in the sugar beet chloroplast. Phenolase may, therefore, have a specific function in the chloroplast, possibly associated with its high cresolase activity.

We thank Mr. G. E. Russell, Plant Breeding Institute, Trumpington, for generous supplies of sugar beet leaves and Dr. R. Hill and Dr. T. Swain for helpful discussions.

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Transmethylation in *Anodonta cygnea*

In a previous survey of the distribution in Nature of betaine-homocysteine-methyl-transferases, these enzymes were found in the livers of all vertebrate animals investigated[1,2]. They could not be detected in any of a number of lower animals examined. The search for the enzymes has since continued, which has led to the discovery of a betaine–homocysteine–methyl-transferase in a member of the Mollusca, the pond mussel *Anodonta cygnea*. The work on which this observation is based will be briefly reported here.

The mussels were harvested in Lake Mälaren and transported in lake water to the laboratory. The livers were excised and homogenized in ice-cold phosphate buffer (0·05 *M*, *p*H 7·8) in a Potter–Elvehjem homogenizer. Methyl donors and homocysteine were added to the homogenate to give incubation mixtures having the compositions shown in Tables 1 and 2. Controls without additions, or containing only betaine or homocysteine, were also prepared. The amount of methionine formed after incubation at 37° C. for 2–4 hr. was estimated microbiologically[3] and in some instances also chemically[4].

Table 1. Formation of Methionine by Homogenates of *Anodonta* Liver in the Presence of the Compounds Listed

Additions	Methionine formed (μgm./mgm. nitrogen per hr.)
None	1·8
0·025 *M* DL-homocysteine	2·9
0·1 *M* betaine	1·6
0·1 *M* betaine + 0·025 *M* homocysteine	8·3
0·1 *M* choline + 0·025 *M* homocysteine	3·0
0·1 *M* betaine + 0·025 *M* homocysteine + 0·1 *M* choline	8·3
0·1 *M* betaine + 0·025 *M* homocysteine + 0·1 *M* dimethylglycine	3·8

Table 2. Effect of Various Methyl Donors
All incubation mixtures contained 0·025 *M* DL-homocysteine

Methyl donor (0·05 *M*)	Relative activity (per cent)
Betaine	100
Betaine methyl ester	100
Betaine amide	0
Carboxyethyl-trimethyl-ammonium-chloride (propiobetaine)	0
Carboxypropyl-trimethyl-ammonium-chloride (butyrobetaine)	0
DL-Carnitine	0
1-Carboxyethyl-trimethyl-ammonium-chloride (α-methylbetaine)	45
Carboxymethyl-dimethylethyl-ammonium chloride	35
Carboxymethyl-methyldiethyl-ammonium chloride	5
Carboxymethyl-triethyl-ammonium chloride*	0*
Dimethylacetothetin†	380
Dimethylpropiothetin†	300
N-trimethyltaurine (taurobetaine)	0

* No ethionine was formed.
† No attempt was made to find optimal conditions for transmethylation from these compounds.

As can be seen from Table 1, the incubation mixtures containing betaine and homocysteine had a higher methionine content at the end of the incubation period than the controls. The presence of homocysteine alone consistently caused the formation of a small but significant amount of methionine, which suggests that the liver of *Anodonta* contains a donor of methyl groups. The addition of choline and homocysteine did not stimulate the synthesis of more methionine than did the addition of homocysteine alone. This indicates that choline is not in itself a methyl donor and that the choline oxidase activity of this tissue is low. The enzyme system causing the formation of methionine from betaine and homocysteine remained in the supernatant fluid after centrifuging homogenates prepared in 0·25 *M* sucrose at 105,000*g* for 60 min. Incubation of the supernatant fluid with ^{14}C-methyl betaine and homocysteine gave rise to radioactive compounds with the same R_F-values as methionine and dimethylglycine in three different solvent systems. The apparent *p*H-optimum of the reaction was near 8·5, which is higher than the optima observed for the methyl transferases of vertebrate livers.

The results in Table 1 also show that dimethylglycine, but not choline, inhibited the synthesis of methionine. Table 2 shows that the methyl donor requirement of the transferase of *Anodonta* liver could be satisfied to a greater or lesser extent by certain betaine derivatives and thetins. It is of interest to notice that the methyl ester of betaine possessed full donor activity, whereas the amide was completely inactive. The length of the carbon chain between the carboxyl group and the nitrogen is apparently of vital importance, as neither propio- nor butyro-betaine was able to serve as donor of methyl groups. Carnitine was also inactive. Both aceto- and propio-thetin, on the other hand, were excellent methyl donors. The introduction of a methyl group in the α-position relative to the carboxyl group in betaine decreased the activity, probably due to steric hindrance.

Replacement of one or two of the N-methyl groups of betaine with ethyl groups gave compounds having approximately 35 and 5 per cent of the activity of unsubstituted betaine. No evidence of ethyl group transfer from these compounds or from 'triethylbetaine' was obtained.

N-trimethyltaurine (taurobetaine), recently isolated from a species of Porifera by Ackermann *et al.*[5], was also tested as a methyl donor but did not seem to have this property. Nor did it act as an inhibitor of the enzyme.

Betaine–homocysteine–methyl-transferase activity could not be detected in any other organ than the liver of *Anodonta cygnea*.

I wish to thank Profs. F. Challenger, G. S. Fraenkel, D. Ackermann and Dr. B. Wickberg for supplying samples of acetothetin, carnitine, 'natural' taurobetaine and synthetic taurobetaine, respectively, and Mr. Nils Walde for help with the synthesis of the other compounds tested as methyl donors. The financial support of the Swedish Medical Research Council and the Knut and Alice Wallenberg Foundation is also gratefully acknowledged.

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Separation of the Hypnotic Potentiating Principles from the Essential Oil of *Acorus calamus* L. of Indian Origin by Liquid–Gas Chromatography

The sedative potentiating activity of the essential oil of *Acorus calamus* L. of Indian and European origin has been reported[1].

It is the purpose of this communication to describe the isolation and identification of the active principle or principles responsible for the above-mentioned pharmacological activity from the essential oil of *Acorus calamus* L. of Indian origin.

In our investigations the volatile oil distilled from *Acorus calamus* L. of Indian origin by Fritzsche Bros., Inc., New York, was used, and liquid–gas chromatography was employed as a technique for the isolation of the individual components. The essential oil was first freed from phenols, acids and carboxylic compounds by the usual chemical procedures[2]. Subsequent to this the majority of other polar components were removed by passing the partly purified oil (75 gm.) through a silica-gel column (40 gm., 100–200 mesh, dried at 110° C.).

The purified oil (50–200 μl.), which exhibited the same pharmacological activity as the crude oil, was injected into an 'Aerograph' liquid–gas chromatography unit (Wilkens Instrument and Research, Inc., Walnut-Creek, California). The column consisted of a 10-ft. length of 1/4-in. stainless steel tubing packed with Johns–Mansville 'Chromosorb' (35–80 mesh) coated with 'Ucon' polar substrate in a ratio 75/25 by weight. The oven temperature was 205° C. and dried helium was used as carrier gas with a flow-rate of 64 ml. per min. measured at the outlet of the apparatus. These conditions were used throughout. Under these conditions the essential oil of *Acorus calamus* L. of Indian origin separated into a number of fractions (Fig. 1).

Four fractions were collected and tested pharmacologically. Fractions U_1 and U_2 appeared to be inactive pharmacologically. Fractions U_3 and U_4 potentiated the activity of pentabarbitone sodium. Fraction U_4 proved to be asarone, 1,2,4-trimethoxy-5-propenylbenzene (calculated C per cent 69·23; H per cent 7·69; found C per cent 69·13, H per

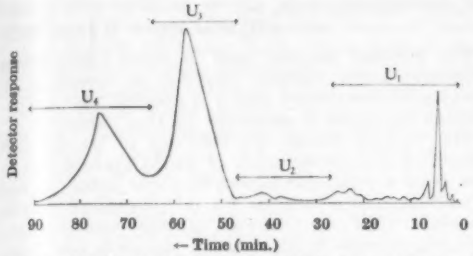

Fig. 1. Liquid–gas chromatogram of 200 μl. essential oil of *Acorus calamus* L. of Indian origin after removing the oxygenated compounds. Column, 'Ucon' polar on 'Chromosorb' 10-ft. × ¼ in. Temperature, 205° C. Flow, 64 ml. of helium per min.

cent 7·43). The compound melts at 58–61° C. (recorded m.p. 61° C. [3]): its picrate melts at 80° C. (recorded m.p. 81–82° C. [4]). Fraction U_3 is a colourless and odourless oil, which when treated with inorganic acids gives the same parasarone as fraction U_4 gives with similar treatment (m.p. 195–200° C., recorded m.p. for parasarone 202–203° C. [5]). After reduction with hydrogen in the presence of palladium charcoal a colourless oil was formed, the retention time of which, under the above-mentioned conditions, is identical with the retention time of the oil derived from fraction U_4 hydrogenated in the same manner. (The hydrogen consumed in both cases is about 1 mole calculated for trimethoxypropenylbenzene.) The ultra-violet spectra of fractions U_3 and U_4 are almost identical. (Absorbancy maxima occur at 253 mμ and 302 mμ.) From these results it can be concluded that the fraction U_3 and fraction U_4 are in *cis–trans* isomeric relationship, as was published earlier[6]—the fraction U_3 proved to be β-asarone.

It is to be noted that re-injection of fraction U_4, which was shown to be asarone, resulted in the appearance of two peaks on the chromatogram (U_4 and $U_{4'}$, Fig. 2, solid line). After several re-injections of the collected asarone, derived from the previous injection, fraction $U_{4'}$ appeared repeatedly and the ratio between the two peaks did not change.

From these observations the conclusion may be drawn that the second peak is not due to a contamination but probably is due to a compound formed during the liquid–gas chromatography procedure. This assumption is supported by another experiment. When pure asarone was kept at 200° C. in a sealed tube under nitrogen for 24 hr., and the mixture formed injected into the column, two peaks, fraction U_4 and fraction $U_{4'}$, also appeared on the chromatogram, but the ratio between the peaks changed significantly compared to the former experiment when pure asarone was injected into the column. The quantity of fraction $U_{4'}$ increased markedly (Fig. 2, dotted line).

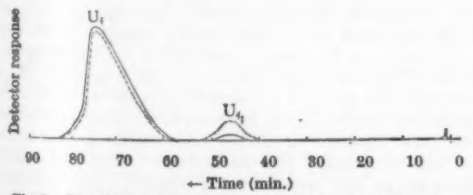

Fig. 2. Liquid–gas chromatogram of 100 μgm. U_4 (solid line) and 100 μgm. U_4 after keeping 24 hr. at 200° C. in a sealed tube under nitrogen (dotted line). Column, 'Ucon' polar on 'Chromosorb' 10 ft. × ¼ in. Temperature, 205° C. Flow, 64 ml. helium per min.

A small amount (about 150 μl.) of the fraction $U_{4'}$ was collected and purified by liquid–gas chromatography. It is a light yellowish odourless oil, which does not crystallize even at temperatures of − 50° C. On treating with inorganic acid a colourless amorphous solid forms, which is different from parasarone. Its ultra-violet spectrum is almost identical with that of asarone and β-asarone. (Absorbancy maximum at 253 mμ and 302 mμ.) The pharmacological activity is different from asarone and β-asarone, in that it possesses not only hypnotic potentiation activity but also sedative activity.

Further studies are in progress to determine the chemical structure of this latter compound. Details of the pharmacological action and chemical nature of these compounds will be published elsewhere.

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Methylglyoxal Formation from Aminoacetone by Ox Plasma

It was previously reported[1–3] that washed suspensions of *Staphylococcus aureus* synthesize aminoacetone from either threonine or glycine and the new aminoketone was isolated in crystalline form. Laver *et al.*[4] and Kikuchi *et al.*[5] have independently demonstrated the formation of aminoacetone by preparations of red blood cells and have shown that the system which forms δ-aminolævulinic acid is responsible for this activity.

I suggested recently[2] that aminoacetone might be involved in a metabolic cycle and postulated that it undergoes deamination to yield methylglyoxal. The present communication is concerned with this aspect of the metabolism of aminoacetone.

Preliminary studies showed that tissue slices of either rat liver or kidney catalysed a slow disappearance of the aminoketone, but so far it has not been possible to demonstrate an unequivocal enzymic attack on aminoacetone using cell-free extracts of these tissues. It was, however, observed that citrated ox plasma catalysed a rapid oxidation of aminoacetone to yield a product which, unlike aminoacetone, gave an immediate reaction with dinitrophenylhydrazine. The enzyme has been purified approximately tenfold by ammonium sulphate fractionation and this purified preparation was used for the subsequent work described here.

To identify the product of aminoacetone oxidation an incubation mixture containing 400 μmoles of aminoacetone (in a final volume of 120 ml.), plasma enzyme, crystalline catalase and phosphate buffer, *p*H 6·2, was shaken for 3 hr. at 37°. At the end of this period the aminoacetone had completely disap-

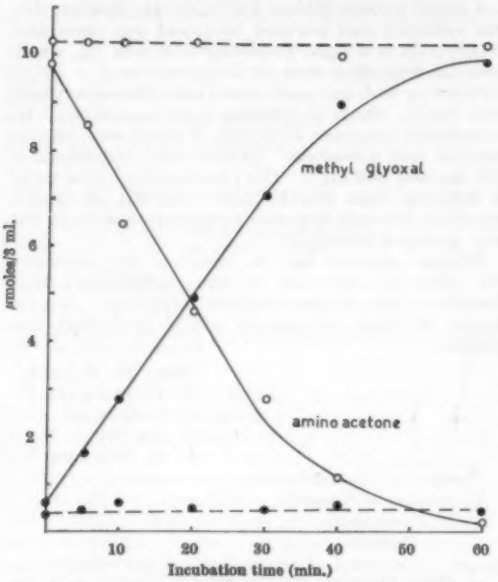

Fig. 1. Broken lines represent a control experiment in which the enzyme was boiled before addition

peared. After deproteinization the extract was passed through a column of 'Zeokarb 225' (*H* form) to remove any amino compounds, and the product isolated from the effluent as the dinitrophenylosazone. A control incubation mixture which was identical, except that aminoacetone was added after incubation, gave no osazone when treated in the same way. The product was recrystallized as orange-red needles from anisole. Yield: 0·056 gm., m.p. 298–300° (decomp.) (lit.[6] 295° decomp.; 299–300° decomp.). Found: C, 41·9; H, 2·9; N, 25·8. Calc. for methylglyoxal 2 : 4-dinitrophenylosazone, $C_{15}H_{12}N_8O_8$: C, 41·7; H, 2·8; N, 25·9 per cent. The isolated product gave the blue-violet colour in alkali characteristic of methylglyoxaldinitrophenylosazone. The identification of the product of aminoacetone oxidation as methyglyoxal was further confirmed by the observation that a partially purified ox heart glyoxalase preparation caused its disappearance, and this was dependent on the addition of glutathione.

Fig. 1 shows the progress of the enzymic reaction with time. Methylglyoxal was formed in amounts equivalent to the aminoacetone disappearing, and no reaction occurred when the enzyme was previously boiled. The aminoketone was quantitatively oxidized and no diminution in the reaction-rate took place until the substrate concentration was reduced to less than 10^{-3} *M*. In these experiments methylglyoxal was measured by a colorimetric method based on that described for pyruvate assay[7].

Manometric studies have shown that in the presence of added catalase, one atom of oxygen was taken up for each molecule of aminoacetone oxidized. In the absence of catalase, the initial rate of oxygen consumption was approximately double that in its presence, but the rate fell off rapidly. However, oxygen uptakes significantly in excess of one atom per molecule of aminoacetone present have been obtained under these conditions, indicating that the following reaction occurs:

$$CH_3.CO.CH_2NH_2 + O_2 \rightarrow CH_3.CO.CHO + H_2O_2 + NH_3$$

The purified enzyme used in these experiments contained amine oxidase activity[8] as measured by benzylamine oxidation and it is possible that aminoacetone oxidation is catalysed by this enzyme.

The present results are believed to be the first unequivocal demonstration of methylglyoxal formation from a substrate known to occur naturally and as such suggest a function for the glyoxalase enzyme system. The distribution of the aminoacetone oxidizing system in mammalian tissues is now being investigated.

Although all the steps of the postulated cycle[2] have now been shown to occur in suitable preparations, it nevertheless remains to be established whether the reactions take place as a metabolic pathway in living organisms. Experiments to this end are being undertaken.

I am indebted to Prof. A. H. Ennor for his stimulating interest in this work and for many helpful discussions.

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Evidence for the Hexose Monophosphate Pathway for Glucose Metabolism in Human Pancreatic β-Cells

The level of glucose in the blood perfusing the islets of Langerhans is thought to regulate the secretion of insulin by the β-cells[1]. At present nothing is known about the mechanism of this regulation or even about the way in which the β-cells metabolize glucose. Studies designed to provide information about these problems are hampered by the fact that the pancreatic islets are rather diffusely scattered throughout the entire exocrine portion of the gland and comprise only about 2 per cent of the total pancreatic tissue[2]. Even if one were successful in obtaining islet tissue free of exocrine pancreas, the β-cells represent only 60–90 per cent of the total cell population[3]. However, in humans there are insulin-producing tumours of the pancreas which are usually composed of only β-cells and such tissue does afford an opportunity to study β-cell metabolism. A ratio greater than unity for the amount of carbon dioxide labelled with carbon-14 derived from glucose-1-^{14}C as compared to that from glucose-6-^{14}C has been taken as evidence for the existence of the hexose monophosphate pathway in the tissue under study[4]. The present report concerns studies using a β-cell tumour and indicates that there is an active hexose monophosphate pathway in these cells (this patient was studied

through the kindness of the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Md.). It is realized that the metabolism in these benign tumours may not be completely analogous to that of β-cells in normal islets.

Paired tissue slices weighing approximately 100 mgm. were incubated in glucose–Krebs Ringer bicarbonate buffer containing 0·5 μc. glucose-1-^{14}C or glucose-6-^{14}C and a final glucose concentration of 250 mgm. per cent. Incubations were done at 37° C. on a Dubnoff shaker under an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide for 90 min. In addition, some flasks also contained 0·01 mgm. of alloxan since this substance is known to destroy β-cells[5]. At the end of the incubation, hyamine was put in the centre well and the carbon dioxide was liberated from the incubation mixture by the addition of 10 *N* sulphuric acid. The hyamine containing labelled carbon dioxide was counted in a liquid scintillation counter using 0·4 per cent diphenyloxazole in toluene as the phosphor. Two control flasks without tissue but with 0·5 μc. glucose-1-^{14}C and glucose-6-^{14}C respectively were also included. For comparison similar experiments were done using rat hemidiaphragm, which is known to be devoid of the hexose monophosphate pathway[6].

The results are shown in Table 1. In slices from the β-cell tumour there were five to six times as many counts in labelled carbon dioxide when glucose-1-^{14}C was present as compared to glucose-6-^{14}C, indicating that the hexose monophosphate pathway was operative. In contrast, the radioactivity in labelled carbon dioxide from the rat muscle was approximately the same for both glucose-1-^{14}C and glucose-6-^{14}C. In addition, there did not appear to be any significant decrease in conversion of glucose to carbon dioxide in the presence of alloxan.

Table 1. Conversion of Glucose-1-^{14}C and Glucose-6-^{14}C to $^{14}CO_2$ by Human Pancreatic β-Cells and Rat Hemidiaphragm

Tissue	Counts/min./gm. in $^{14}CO_2$ from Glucose-1-^{14}C		Glucose-6-^{14}C	
β-cells	16,030	(2)	3,885	(2)
β-cells + alloxan	19,225	(2)	3,650	(2)
Rat hemidiaphragm	9,325	(2)	9,070	(2)

Number of determinations in parentheses

The full significance of the presence of an active hexose monophosphate pathway in β-cells cannot be ascertained although one can make some interesting inferences. Recently, the role of this pathway as a source of reduced triphosphopyridine nucleotide has been emphasized, and it has been suggested that it is of importance in many synthetic processes[6]. It has also been reported that stimulation of this pathway can lead to increased protein synthesis[7]. It is quite conceivable that the synthesis and secretion of insulin, which appears to be regulated in some manner by the blood glucose concentration, are thus facilitated by glucose metabolized via the hexose monophosphate pathway.

The finding that alloxan had no apparent effect on the conversion of glucose to carbon dioxide was somewhat unexpected. It may be that the dose of alloxan was too small or that alloxan, itself, is not toxic but that a metabolic product in the body is responsible for the β-cell damage. Other possibilities are that alloxan requires more than the 90-min. incubation period to disrupt β-cell metabolism or that the effects of this drug are not exerted on glucose catabolism but rather on some other essential cellular process[8]. It is also conceivable that β-cells in islet cell tumours are more resistant to alloxan than β-cells in normal islets.

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ANIMAL PHYSIOLOGY

A Peanut Factor for Hæmostasis in Hæmophilia

It is known that there are unpredictable apparent remissions of clinical symptoms enjoyed by hæmophiliacs; but these remissions have not been correlated with any influences such as time of year, food eaten, weather conditions, other diseases, or physical condition of the patient. The lack of 'antihæmophilic factor' associated with classic hæmophilia has been attributed[1] to a hypothetical block during the metabolic synthesis of the factor resulting from the action of the mutant gene of hæmophilia, such as occurs in the nutritional mutants of *Neurospora* and in eye pigment development in certain mutants of *Drosophila*[2]. In the cases of *Neurospora* and *Drosophila*, and other organisms studied, the addition of the substances the synthesis of which is blocked genetically results in normal metabolism. If this is the case in hæmophilia, it may be possible to supply, by mouth or injection, the unavailable material necessary for normal production of the hæmostatic substance lacking in hæmophiliacs. However, the few instances of success of various supplements, such as vitamins E and B_{12}, X-rays, and œstrogen, have not been confirmed[3–7].

One of us (H. B. B.) suffers from antihæmophilic factor hæmophilia, and in the past has been able to perform his duties with the help of periodic transfusions of fresh blood or infusions of lyophilized plasma following a hæmophilic attack. Various foods likely to overcome the hypothetic metabolic block by furnishing substances in the antihæmophilic factor pathway have been ingested in large daily quantities (blood pudding, liver, raw milk, eggs) without causing any reduction in severity of the disease. In May 1957, however, there seemed to have occurred a rapid loss, following consumption of a large handful of roasted peanuts, of tenderness (indicating a stoppage of bleeding) in a knee with an active hæmatoma. This loss in tenderness occurred without other therapy.

Since then, peanuts in all available forms (commercial peanut-butter, roasted, or raw peanuts) have been ingested on the appearance of each hæmophilic

attack, and in each instance the clinical symptoms were relieved in one or two days.

During a period of more than two years, when peanuts, peanut flour, or a concentrate prepared from peanut flour has been in use, it was necessary only once to use lyophilized plasma to control a bleeding episode. This bleeding occurred in the lower pharynx, making ingestion impossible. Whenever peanut products are avoided, the tendency to bleed recurs, but yields to re-establishment of peanut regimen.

The relief of clinical symptoms brought about by peanut material is not correlated with reduction of capillary blood clotting time measured in glass capillary tubes, or of venous blood clotting time measured by the Lee–White method. No other attempts were made to measure any possible effects *in vitro*. In spite of the unreliability of the capillary tube method of measuring clotting time, the deepest punctures during periods of ingestion of peanut material have always healed without special treatment or rebleeding. The same normal healing has occurred in all cases of skin wounds occurring during the ingestion of peanut material.

Efforts have been made to concentrate the active factor. Peanut flour, prepared on extracting lightly roasted peanuts with hexane, and the crude extracted oil were each tested separately. The hæmostatic effect seems to be confined to the flour. It was found by Boudreaux, and by a second antihæmophilic factor hæmophiliac volunteer (aged fifty-three), that clinical symptoms are apparently relieved in one or two days with doses of 180 gm. of the flour per day. A third volunteer (aged five) has had relief from bleeding while taking flour or screw-pressed extracted meals. Boudreaux is at present effectively using screw-pressed extracted meal as a prophylactic.

The active factor is removed from peanut flour by extraction with 90 per cent ethyl alcohol. The residue was ineffective in relieving clinical symptoms, whereas the alcohol-extracted material was apparently effective in one or two days in doses of 14 gm. (the equivalent of 1 lb. of peanuts) per day, as indicated in tests carried out by Boudreaux and a fourth volunteer (aged eighteen). In the case of the fourth hæmophiliac, relief was obtained with the concentrate in two days, when he had failed to respond to 9 plasma infusions administered over a period of 3 weeks. A finger which was badly bruised in a motor-car door during the period of ingestion of peanut concentrate required no special treatment. During another bleeding episode, 12 infusions of fresh plasma given to this fourth volunteer in 10 days did not stop bleeding from the kidneys. Plasma was discontinued, and two days later the alcohol-extracted material was administered orally in 5-gm. quantities every 6 hr. No blood appeared in the urine on the second day. The volunteer was discharged from the hospital three days later.

It is unlikely that enough antihæmophilic factor was produced on ingestion of the concentrate to cause clinical relief, but enough to affect 'clotting time'. Enhanced thrombocyte action alone may be sufficient for hæmostasis without fibrin production[8]. Another possibility is that the peanut factor hastens capillary healing or improves capillary integrity without effect on blood hæmostatic reactions[9]. Current tests on hamsters in our laboratory suggest that vaso-constriction is enhanced by ingestion of the concentrate.

It is hoped that these limited observations will incite the interest of medical research to help determine the general occurrence of this phenomenon. Meanwhile, efforts are continuing to concentrate and identify the active factor, and to develop a bioassay on laboratory animals.

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Distribution of Tryptophan in the Brain

It is well established that the central nervous system of vertebrates contains a substance which is biologically and chromatographically indistinguishable from 5-hydroxytryptamine. The tissues richest in 5-hydroxytryptamine are the diencephalon and mesencephalon, with little or none in the cerebellum or white matter. In the course of paper chromatographic studies on the distribution of 5-hydroxytryptamine in the brain of vertebrates, a second indole substance was noted and afterwards identified as the amino-acid, tryptophan. The distribution of tryptophan did not always follow closely that of 5-hydroxytryptamine, and this result may be of importance since it is believed that 5-hydroxytryptamine is formed by the decarboxylation of 5-hydroxytryptophan, an amino-acid formed from tryptophan.

Acetone extracts of tissues from freshly killed animals were subjected to paper chromatography in four solvents: (1) 8 per cent (w/v) sodium chloride solution, (2) *iso*propanol/ammonia/water (20 : 1 : 2), (3) *n*-propanol/water (3 : 1), (4) *n*-butanol/acetic acid/water (4 : 1 : 5). The identity of tryptophan was established chemically by its R_F values in the different solvents and by its colour reactions with ninhydrin, Ehrlich's reagent, Pauly's reagent and *N.N.C.D.* reagent. Further, superposition of the tryptophan spot given by the tissue extracts and that given by tryptophan added to such extracts was obtained and a similarity of the colour reactions was observed.

The brain of dog, cat, mouse, guinea pig, rat, rabbit, pigeon, hen and gull contained between 1 and 3 μgm. tryptophan per gm. of fresh tissue, and these concentrations were between 3 and 10 times the corresponding concentrations of 5-hydroxytryptamine. Other tissues in the body such as the spleen, gut and blood also contained 3–10 times as much tryptophan as 5-hydroxytryptamine.

When the various parts of the brain of the dog, cat and rabbit were dissected out and assayed for their tryptophan content, the richest sources were found to be the pons and hypothalamus. The mid-brain medulla and cerebellum contained appreciable quantities whereas the cerebral hemispheres and spinal

Table 1. Estimates of Tryptophan and 5-Hydroxytryptamine (μgm./gm. Tissue) in Dog Brain

Part of brain	Tryptophan	5-Hydroxytryptamine
Cerebral hemispheres	0·1	0·01
Hypothalamus	5–40	0·40
Mid-brain	2–8	0·35
Cerebellum	1–4	0·02
Pons	1–20	0·05
Medulla	2–5	0·25
Spinal cord	0·2	0·02

cord showed only traces. The results for the dog are shown in Table 1 together with estimates of 5-hydroxytryptamine for the corresponding areas. Inspection of the figures shows that the tryptophan content of three areas—the hypothalamus, cerebellum and pons—was about 100 times that of 5-hydroxytryptamine whereas the ratio was only 3–10 for the other parts. A similar result has also been found for cat and rabbit brain. The reason why the hypothalamus, cerebellum and pons are relatively rich in tryptophan and poor in 5-hydroxytryptamine is not clear at present.

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Resting Potentials in Isolated Frog Sartorius Fibres at Low External Potassium Concentrations

In earlier experiments referred to by Conway[1,2], it was shown that the resting potential (E) of isolated frog sartorii fibres corresponded to the theoretical values as determined by the relation :

$$E = \frac{RT}{F} \ln [K]_o/[K]_i = \frac{RT}{F} \ln [Cl]_i/[Cl]_o \qquad (1)$$

provided the conditions were such that a 'balanced state' (state of zero net flux) existed for the sodium and potassium ions.

The validity of the Conway theory, from which such an equation may be derived, has been well tried and supported by Küsel and Netter[3], by Mond[4], Adrian[5], Hodgkin[6]; Hodgkin and Horowitz[7]; also for mammalian skeletal muscle by Pillat, Kraupp, Giebisch and Stormann[8].

Here various potential measurements using the micro-electrode technique[9] were carried out[10] with fibres of frog sartorii which had been immersed overnight at 0–3° C. in Ringer–Conway fluid with constant sodium concentration, and potassium values up to very high concentrations, as great as 200 mM [11].

A series of determinations was also carried out with the impermeant anion, acetate, replacing all the chloride in the external solution, potassium being substituted for sodium up to high levels, the solution being thus maintained isotonic.

In both these types of experiment the theoretical potential with respect to the potassium ion was obtained above external values greater than 10 mM. Below this level the potential tended to fall below the theoretical figure, corresponding with the observations of Adrian, and of Hodgkin and Horowitz.

Immediate determinations of the resting potential of isolated fibres using Ringer–Conway fluid with normal plasma potassium level of 2·5 mM gave a value of 92·5 ± 0·47 mV., the theoretical figure being 100·1 mV. (the 'fibre water' potassium was 133 mM for the group of frogs used here). Adrian obtained similar results, the average fibre water potassium of his frogs being 139 mM.

The points on the curve of log [K] against E were shown by Hodgkin and Horowitz to be reasonably well fitted at the low potassium levels of 1–10 mM and 20° C. by the relation :

$$E = 58 \log \frac{[K]_o + 0{\cdot}01\,[Na]_o}{[K]_i + 0{\cdot}01\,[Na]_i} \qquad (2)$$

Above an external concentration of potassium of 10 mM, this equation gives results practically identical with equation 1. The fall below the potential-levels from equation 1 at external values of potassium less than 10 mM could be assigned initially to either of two causes. First, to the existence of a sodium–potassium linked pump which would result in an equation of the second type given above.

It could also arise if a 'balanced state' with respect to sodium and potassium ions did not in fact exist for isolated sartorii fibres with the external potassium concentration less than 10 mM. These two views are also referred to by Hodgkin and Horowitz[7], with the conclusion that their published observations are consistent with either of them.

To examine the question here the observation of Carey and Conway[12] was utilized that with plasma as the external medium the permeability of the fibres to sodium ions was retained closer to the normal.

Plasma was prepared by bleeding through an aortic cannula into a heparinized tube. The blood was centrifuged at once and the plasma analysed for its potassium content, suitably diluting or adding potassium chloride if necessary to give 2·5 mM.

Table 1

External fluid	External potassium concentration	Resting potential observed mV.	Resting potential theoretical mV.
Ringer–Conway	2·5	92·5±0·47	100·1
Plasma	2·5	99·2±0·7	100·1

Such results show that the hypothesis of a sodium–potassium linked pump is unnecessary with respect to the potential measurements for frog skeletal muscle, and is not the explanation of the fall from the theoretical potential values from equation 1, for isolated muscle at low external potassium-levels.

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Neutralization of Human Serum and Pituitary Thyrotropic Fractions with Antisera to Bovine Pituitary Thyrotropin

Antisera to pituitary growth hormone have been shown to possess a high degree of species specificity[1]. This was demonstrated by precipitin and by neutralization experiments, in which the antiserum to human growth hormone was active against human and monkey growth hormone, but not against growth hormone derived from other species.

Experience over the past years with antisera to a thyrotropic preparation of beef pituitary has revealed that the antisera do not possess great specificity when tested against thyrotropin from several other species[2]. In the present work, the observations have been extended to man. Antibovine thyrotropin sera have been found to neutralize the thyrotropic activity of fractions prepared from human pituitaries and from two individual sera, one from a patient with classical Graves's disease and the other from a healthy euthyroid subject.

Rabbits were immunized by the method of Freund[3], using a commercial bovine thyrotropin preparation in current clinical use ('Thytropar') as the antigen. Details of the methods employed and the results from precipitin and neutralization studies with the parent and other more purified thyrotropic extracts are being published elsewhere. The antibody-containing globulin fraction was concentrated from the antisera and from normal rabbit globulin by sodium sulphate precipitation[4].

The serum fractions were prepared by the method of Bates *et al.*[5]. The human pituitary preparation, not at the final stage of purification, was made by Heideman and Bakke[6] from pooled glands obtained at autopsy and was obtained through their courtesy. The active material contained 10 mμ/c.c.

The hormone to be neutralized and normal serum or antiserum, or the globulin preparation made from antiserum or normal serum, were mixed and kept overnight at 4° C. In one experiment (Exp. 234), previous absorption of both normal and antiserum with bovine serum was conducted prior to mixing. Assay for thyrotropic activity was performed by a slight modification of the method of McKenzie[7]. The iodine-131 content of the blood was measured before and 2 hr. after intravenous injection of the test sample. In each assay, saline, the human pituitary or serum preparation mixed as above with serum or globulin preparation of serum, and two dose-levels of a standard preparation of beef thyrotropin were assayed concurrently. Four mice were employed for each group.

It is evident from Table 1 that there was complete neutralization of human thyrotropin from both pituitary (Exps. 138, 230 and 234) and serum (Exps. 208 and 188) sources by the several anti-bovine thyrotropin sera used. No neutralization occurred when normal rabbit serum was employed. In Exp. 230, the

Table 1. Neutralization of Thyrotropin from Human Pituitary and Serum by Anti-beef Thyrotropin Serum

Exp. No.	Test material	No. animals	Dose: TSH	Dose: Serum	Mouse assay (per cent of initial blood radioactivity at 2 hr.) Mean ± S.D.	P value*	Remarks
138 and 140	Saline	10			97 ± 26		
	Human TSH	10	0·5μ		267 ± 133		
	Human TSH + normal rabbit serum globulin	6	0·5μ	3·0 mgm. (0·15 c.c. equiv.)	337 ± 101		Antiserum to relatively crude bovine thyrotropin
	Human TSH + antiserum globulin	4	0·5μ	2·0 mgm. (0·1 c.c. equiv.)	113 ± 44	$P < 0.001$	
230	Saline	4			68 ± 19		
	Human TSH	4	0·5μ		278 ± 90		Antiserum to highly purified bovine thyrotropin, 25 μ/mgm. (ref. 10)†
	Human TSH + pre-immune rabbit serum	4	0·5μ	0·5 c.c.	219 ± 51		
	Human TSH + post-immune rabbit serum	4	0·5μ	0·5 c.c.	106 ± 31	$P < 0.001$	Pre-immune serum obtained from same rabbits as post-immune
		4					
234	Saline	4			81 ± 11		
	Human TSH	4	0·25μ		170 ± 35		Antiserum to highly purified bovine thyrotropin, 25 μ/mgm. (ref. 10)†
	Human TSH + normal rabbit serum	4	0·25μ	0·5 c.c.	147 + 44		
	Human TSH + antiserum	4	0·25μ	0·5 c.c.	73 ± 11	$P < 0.001$	Absorption of both normal and anti-serum with bovine serum before experiment
208	Saline	4			94 ± 16		
	Bovine TSH	4	0·1μ		325 ± 36		Antiserum to relatively crude bovine thyrotropin; absorption with human hypopituitary serum before experiment
	Toxic diffuse goitre serum preparation‡	4	7·5 mgm. (1 c.c. equiv.)		297 ± 64		
	Toxic diffuse goitre serum preparation + antiserum globulin	4	7·5 mgm. (1 c.c. equiv.)	3·1 mgm. (0·4 c.c. equiv.)	92 ± 18	$P < 0.001$	
188	Saline	4			129 ± 50		
	Human TSH	4	0·1μ		240 ± 39		Antiserum to relatively crude bovine thyrotropin
	Normal human serum preparation	4	0·05 mgm. (0·01 c.c. equiv.)		234 ± 61		
	Normal human serum preparation + normal rabbit serum globulin	4	0·05 mgm. (0·01 c.c. equiv.)	10·0 mgm. (1 c.c. equiv.)	227 ± 30		
	Normal human serum preparation + antiserum globulin	4	0·05 mgm. (0·01 c.c. equiv.)	10·0 mgm. (1 c.c. equiv.)	153 ± 26	$P < 0.001$	

* P values calculated for difference between the means of values for thyrotropin preparation plus normal serum or antiserum, except in experiment 208, where difference was calculated from test serum fraction alone.

† Experiment confirmed with an antiserum to a preparation containing 25 μ/mgm. kindly provided by Dr. John G. Pierce, University of California.

‡ Insufficient preparation for test with normal rabbit serum globulin.

normal serum was obtained from the same rabbit in which the antiserum was induced. In Exp. 234, the normal serum was absorbed with normal bovine serum in the same manner as the antiserum. The failure of the bovine serum absorbed antiserum to neutralize the thyrotropin excludes the possibility of precipitation of hormonal activity along with non-specific proteins. The failure of the pre-immune and normal rabbit sera to neutralize excludes the presence of neutralizing substances in serum prior to immunization.

The nature of the material in human serum which induces a thyrotropic response, especially in Graves's disease, has not been as yet clearly identified as thyrotropin. Adams and Purves have observed that the release of iodine-131 from the thyroid is delayed following injection of thyrotoxic serum compared to standard preparations or to hypothyroid serum[8]. They suggested that possibly the thyrotropin in the serum of Graves's disease patients may be abnormal. This observation in the guinea pig has been confirmed in the mouse[7].

More recent work by McKenzie indicates that the material in hyperthyroid sera will cause histological activation of the mouse thyroid, an increased uptake of iodine-131, and an increase in level of protein-bound iodine[9]. This suggests that the active material in the serum is a thyrotropin. The delay before activation of the thyroid, however, by such a sera could also imply that the serum factor is not a thyrotropin, but only acts indirectly to cause the mouse to release its own thyrotropin.

As stated, the present experiments reveal that the thyrotropic effect of the thyroid activating substances in normal and in hyperthyroid serum were equally well neutralized by an antithyrotropic serum. This suggests that the active material in both is a thyrotropin which is closely similar if not identical to that from the pituitary. It is not proved that endogenous mouse thyrotropin was not secreted and then neutralized by the injected antiserum. However, the extent of the dilution of the antiserum in the circulation of the mouse makes this possibility less likely, as does the short time, 2 hr., between injection and evidence of thyroid response. This allows only a very brief interval for the diluted antibody to have reacted with endogenous thyrotropin were it secreted.

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A Substance in Lingcod Liver Oil which prevents Hypercholesterolæmia in Cholesterol-fed Chickens

The serum cholesterol-level in some animals is elevated above the normal value by the addition of cholesterol to the diet. In certain species, such as the chicken, cholesterol by itself brings about this increase; but in other species, exemplified by the rat, the presence of cholic acid is also required. The degree of hypercholesterolæmia is altered when certain vegetable and fish oils are added to the diet; but the observed effect of any one oil is dependent on the species of animal under investigation. De Groot and Reed[1] showed that cod liver oil and corn oil prevented the increase in plasma cholesterol in rats due to dietary cholesterol and cholic acid. Dam *et al.*[2] investigated the effect of dietary cod liver oil and linseed oil in cholesterol-fed chickens, and found that both oils increased the hypercholesterolæmia caused by the dietary cholesterol. Similar experiments on chickens were performed by Wood and Biely[3] using corn oil, tallow and various fish oils. Corn oil, tallow and herring oil were observed to increase the hypercholesterolæmia; but lingcod liver oil and halibut liver oil completely prevented the appearance of hypercholesterolæmia. Ratfish liver oil, dogfish liver oil and basking shark liver oil were intermediate in effect. In view of the diverse effects of the oils observed in the chicken studies, further investigations were carried out to obtain additional information about the mode of action of the oils. Corn oil, herring oil and lingcod liver oil were chosen for the investigation.

The oils were saponified with ethanolic potassium hydroxide, and the soap solutions were extracted several times with ethyl ether to obtain the unsaponifiable material. The soap solutions were then acidified with hydrochloric acid and the free fatty acids extracted with ethyl ether. The extracts containing the unsaponifiable fractions and the free fatty acids were washed and the solvent removed on a water-bath at 70° C. and under a stream of nitrogen. The oils and their components were added to a diet containing 1 per cent cholesterol. This diet was fed to 2-weeks old New Hampshire chickens for 9 days, after which time blood samples were taken and the serum cholesterol concentrations determined. The oils were fed at a 10 per cent level in the diet and the unsaponifiable substances and the free fatty acids were added in amounts approximating to that obtained from an equivalent amount of oil.

As observed previously, lingcod liver oil prevented the hypercholesterolæmia caused by the dietary cholesterol, and herring oil and corn oil stimulated the rise in serum cholesterol concentration (Table 1). The free fatty acids from all three oils markedly increased the degree of hypercholesterolæmia, whereas

Table 1. Effect of Various Oils and Fractions from these Oils on Serum Cholesterol-Levels in Cholesterol-fed Chickens

Addition to the cholesterol-containing diet	Serum cholesterol (mgm./100 ml.)
None	192 ± 12*
Corn oil	784 ± 173
Herring oil	648 ± 133
Lingcod liver oil	130 ± 31
Corn oil fatty acids	793 ± 144
Herring oil fatty acids	618 ± 215
Lingcod liver oil fatty acids	702 ± 158
Corn oil unsaponifiables	193 ± 21
Herring oil unsaponifiables	237 ± 34
Lingcod liver oil unsaponifiables	119 ± 17

* Each value is the mean for four samples of serum ± the standard deviation. Each sample contained the serum from four chickens.

the unsaponifiable material from herring oil and corn oil produced only a slight increase or none at all. The unsaponifiable portion of lingcod liver oil, however, completely prevented the rise in serum cholesterol-level.

It was concluded that the ability of lingcod liver oil to prevent hypercholesterolæmia in cholesterol-fed chickens depends on the presence of some compound or compounds in the unsaponifiable material of the oil. At the present moment the active material has not been identified; but the possibility exists that it may be vitamin A or perhaps a sterol-type compound which acts in a similar manner to cholestanol. The latter compound was shown[4] to prevent effectively the appearance of hypercholesterolæmia induced in chickens by cholesterol feeding.

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SEROLOGY

A Starch Gel Immunoelectrophoretic Analysis of Human γ-Globulin demonstrating the Inter- and Intra-Specific Variation of Organisms

An age-dependent, phenotypic variation in the antigenic composition of human γ_2-globulin has been reported[1–3]. Chicken antisera to preparations rich in γ-globulin (for example, *FrII*) were used to examine human sera by the starch gel immunoelectrophoretic method of Poulik[4] as modified by Goodman[3,5]. Certain of these antisera detected in many instances more than one component in that portion of the γ-globulin which moves backward from the origin in starch gel electrophoresis, that is, in the γ_2 fraction of serum. Recently, as the point of departure for the present work, non-human primate sera were also examined by some of the antisera. It was reasoned that, since the evolutionary continuum leads from the intra- to the inter-specific variation of organisms, the serum antigenic specificities which differ between human beings would fail to have derivative counterparts in the sera of the non-human primates. A considerable body of data illustrating the applications of this reasoning has now been gathered, and several examples of the data are presented in Figs. 1 and 2.

The immunoelectrophoretic reactions depicted in Fig. 1 show that the absorption of chicken antiserum with rhesus monkey serum selectively lowers the antibody concentration to the predominant antigenic groupings of human γ-globulin and thus permits a visualization of those groupings held by only a fraction of the γ_2-globulin molecules, a fraction which varies markedly between human beings, and which appears to occur more richly in adult sera than in prepuberty sera[2,3]. Note that such a fraction could be detected in the 'positive' human serum pool (made up of several adult sera) after antisera *C*-341 and *C*-342 were absorbed with the rhesus serum. The fragmented γ_2-globulin precipitin arc signifying a positive reaction was observed with single serum specimens as well as with the 'positive' pool. (An example of such a result is photographically reproduced in Fig. 1*E*.) On the other hand, a large number of the serum specimens from adults and children, as well as the 'negative' pool (made up of several prepuberty sera), failed to yield the second γ_2 reaction with these two reagents.

The results obtained with the antisera, *C*-79 and *C*-82, present a somewhat different picture, for these antisera each contained, as compared to *C*-341 and *C*-432, antibody directed to a large percentage of the spectrum of antigenic groupings showing an inter-specific variation between man and monkey. The additional γ_2 are developed with the 'negative' pool as well as with the 'positive' pool. However, Fig. 1 (*C* II and *D* II) does show that the absorption with the rhesus serum left a more potent reaction for the variant γ_2 fraction of the 'positive' pool than for that of the 'negative' pool.

A number of human and non-human primate sera have now been surveyed for the presence or absence of the additional γ_2 reaction by unabsorbed *C*-79 and by *C*-82 after an absorption with a small quantity

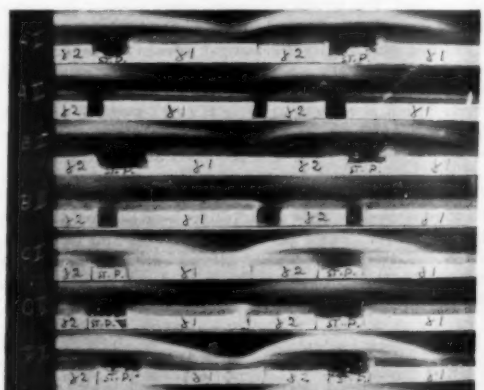

Fig. 1. Photographs of the reactions by double diffusion in agar gel of chicken anti-human γ-globulin sera against human serum separated by starch-gel electrophoresis. From *A* to *D* each reaction tray contains at its bottom the γ-globulin portions from the starch-gel separation of a 'negative' human serum pool and a 'positive' human serum pool (see text). Four different antisera are represented: *C*-341 in *B*, *C*-82 in *C* and *C*-79 in *D*. In each instance the reactions of the antiserum before absorption (I) are compared to those obtained after an absorption (II) with an aliquot of rhesus monkey serum estimated to contain 198 mcgm. γ-globulin per ml. of antiserum. In *E* the rhesus absorbed *C*-341 reacts against the γ_2-globulin portions of four different adult human sera. The second γ_2-precipitin arc is marked in samples 295 and 296 but weak to negative in samples 288 and 290. The non-specific lateral band that develops in the agar near the surface of the starch gel block can be so recognized and thus discounted in assessing the specific antibody–antigen reactions. γ_2, The γ_2-globulin portion of the starch gel block; *st.p.*, starch paste, in which the serum sample was originally inserted; γ_1, the γ_1-globulin of the starch gel block

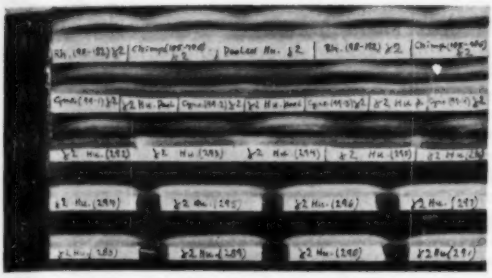

Fig. 2. Photographs of immunoelectrophoretic results obtained on surveying sera from the rhesus monkey (*Rh*), the cynomolgus monkey (*Cyno*), the chimpanzee (*Chimp*), and the human (*Hu*) for the presence or absence of a 'minor' γ_2-globulin antigen as detected by absorbed antiserum *C*-82 reacting against the γ_2-globulin portion (γ_2) of the starch gel blocks. For the *A* trays, *C*-82 was absorbed per ml. with an aliquot of 'negative' human serum estimated to contain 125 mcgm. of γ-globulin. A 'minor' γ_2-reaction occurring underneath the 'major' precipitin reaction can be seen only for the human samples. In the *B* trays, *C*-82 was absorbed per ml. with 410 mcgm. of γ-globulin of the appropriate human serum. In the two trays represented positive reactions can only be seen for human sera 294, 295 and 296. It is of interest that 294, 295, 296 and 297 are *Gm*-like (−) samples and 288, 289, 290 and 291 are *Gm*-like (+) samples. (The absorbed *C*-82, however, was not the best reagent of those tried for distinguishing *Gm*-like (−) samples from *Gm*-like (+) samples.)

of the 'negative' human serum pool which by weakening the predominant precipitin arc allowed the additional γ_2 arc to be fully revealed. All the adult human sera tested (14 samples with *C*-79 and 39 with *C*-82) yielded this additional reaction, though to varying degrees, but none of the non-human primate sera did. The survey has encompassed sera from 13 chimpanzees, a gibbon, 16 rhesus monkeys, 15 cynomolgus monkeys, 2 baboons, 2 mandrills, a langur, 2 spider monkeys, and a capuchin monkey. Examples of the results obtained in the survey are presented in Fig. 2*A*. Also note (Fig. 2*B*) that when *C*-82 was so absorbed with the appropriate serum pool that it could no longer yield in immunoelectrophoresis its 'major' γ-globulin precipitin arc, its 'minor' γ_2-precipitin arc (the one missing from non-human primates) was no longer developed by all human adult sera, but only by some.

The relationship of the antigenic components detected by such chicken antiserum reagents to the *Gm* groups[6] is now under investigation. For this purpose Dr. Grubb has kindly furnished 6 *Gm* (*a* +) sera and 6 *Gm* (*a* −) sera. Similarly, Dr. Steinberg has kindly furnished 6 of his *Gm*-like (+) sera which are present in some Negroes and rare or absent in whites, and 6 of his *Gm*-like (−) sera[7]. Although none of the reagents has distinguished the *Gm* (*a* +) group from the *Gm* (a −) group (each yielding positive and negative reactions with both groups), some of these reagents did distinguish the *Gm*-like (+) group from the *Gm*-like (−) group in that they reacted only with *Gm*-like (−) samples and not with the *Gm*-like (+) samples. The interrelationships of such γ-globulin specificities may prove to be so complex as to argue for the thought that genetic information inherited through a number of gene loci rather than just through a *Gm* locus[8] goes into shaping the antigenic architecture of a γ_2-globulin molecule. In this regard, an answer is being sought to the crucial question of whether there is in addition to the variant antigenic configurations detected an overall antigenic resemblance between the above 'major' and 'minor' fractions of the γ_2-globulin of human serum.

This investigation was aided in part by research grants from the National Institutes of Mental Health and the (American) National Multiple Sclerosis Society. I am indebted to Dr. Albert B. Sabin at the Children's Hospital Research Foundation in Cincinnati, and to Dr. Sidney Gains, chief of the Department of Microbiology at the Walter Reed Army Institute of Research, for providing me with the chimpanzee serum, and to Dr. G. D. Brigham, director of the Biological Division at Parke, Davis and Co., for the rhesus and cynomolgus monkey sera. I am also grateful to Dr. George B. Rabb, curator of the Chicago Zoological Park, for the many samples of primate sera. The expert assistance of Emily Poulik, Roger Gudobba, and Rosemarie McBride is greatly appreciated.

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Changes in Outdated Human γ-Globulin Preparations

THE fate of antibodies in normal human γ-globulin preparations during storage was studied by Gerlough[1], who succeeded in achieving surprising results: the half-time of diphtheria antitoxin in the preparation was 23 years—under strict storage conditions even 40–69 years. Tiselius electrophoresis and ultracentrifugation did not reveal cleavage or denaturation of the protein molecule.

We have studied a number of outdated normal human (plasmatic) γ-globulin preparations prepared by Cohn's classical methods 6 and 9 as well as by other methods. The products were stored at + 4° C. for 3–6 years in the form of sterile solutions. No difference was observed in the contents of diphtheria antitoxin compared with fresh products, though immunoelectrophoretically two atypical fractions besides the normal precipitation line were noticed. Of these atypical fractions one possessed a mobility lower (called γ_X) and the other higher (γ_Y) than γ-globulin. The γ_Y component appears sooner than the γ_X one, so that some of the preparations studied possessed only the γ_Y component and some both of them. Both fractions are not precipitated by antisera with saturated anti-γ-globulin antibodies. Fig. 1 presents two γ-globulin products prepared four years ago by Cohn's classical method. These products were originally absolutely homogeneous. When studying the γ-globulins prepared from materials containing hæm and hæmoglobin a similar phenomenon was observed, and even much sooner after the preparation of solution: the time after which the fractions begin to appear is in case of plasmatic preparations a matter of years—in case of placental preparations of the order of months.

Plasmatic and placental products stored one year at + 4° C. were compared by quantitative precipita-

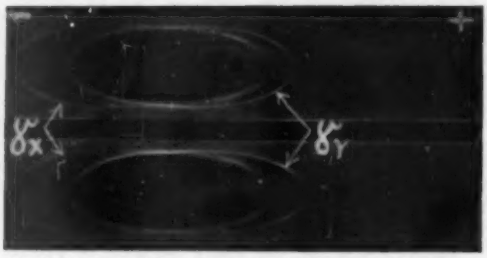

Fig. 1. Immunoelectrophoresis of γ-globulin prepared according to Cohn, aged for 3 yr. at + 4° C.

tion with antihuman antiserum by the capillary tube technique with the following results: plasmatic γ-globulins not yet split were precipitated in a considerably higher dilution (1 : 2,000–4,000) than placental preparations—already split (1 : 32–128). This fact indicates a partial destruction of groups determining the species specificity of the protein in the course of splitting.

The changes in γ-globulins during storage result from a slow proteolytic process. Fractions having identical electrophoretic mobility were obtained by trypsin-, plasmin-, and papain-digestion of a standard γ-globulin. No such manifestations were observed when pepsin-digestion was applied.

The γ-globulin fractions resulting either from storage or from tryptic-digestion can be partially separated by ammonium sulphate precipitation. In Fig. 2 are (from above downwards) immunoelectropherograms of the original outdated product prepared from hæmolysed materials, and fractions appearing after 25 per cent, 25–30 per cent, 30–35 per cent, and 35–50 per cent saturation. At more than 50 per cent only the γ_X component is precipitated. The fraction between 35 and 50 per cent contains a mixture of γ_X and γ_Y component without the original γ-globulin. It is interesting that these two proteins behave as immunologically different antigens, judging from the site of arc-contact according to Ouchterlony's principle[2].

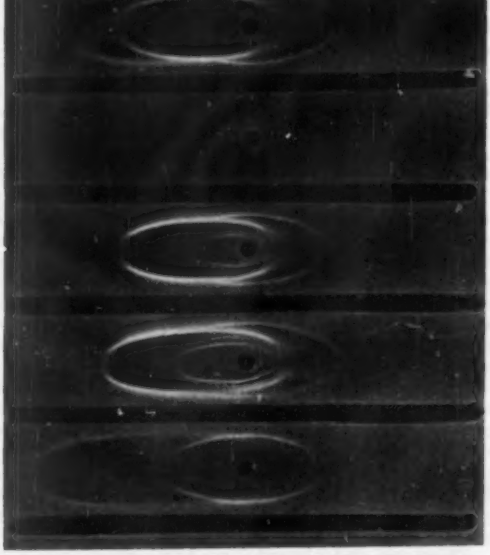

Fig. 2. Ammonium sulphate fractionation of spontaneously split γ-globulin from hæmolysed material

Among the products studied was also a sample of γ-globulin prepared experimentally by Lever's modification of Cohn's method 10 from placentæ[3]. This preparation contained in the final 16 per cent solution approximately 0·3 per cent of sodium tartarate as a remainder from the production technique. In this product both the above-mentioned fractions appeared in the course of a few months accompanied simultaneously by the formation of a considerably large quantity of small needle-like crystals showing a pearly glossy colouring after shaking (Fig. 3). Immunoelectrophoresis of isolated, washed and recrystallized particles revealed that their protein component was formed by the atypical γ_Y fraction. The structure of the crystals and the role of the other ingredients present in the solution will be studied in detail in the future. By ultracentrifugation of the original, spontaneously storage-split product two fractions were isolated, one having S_{20} 4·8, the other 8·2; the solution of the crystalline fraction was homogeneous in the ultracentrifugation, having S_{20} 4·4.

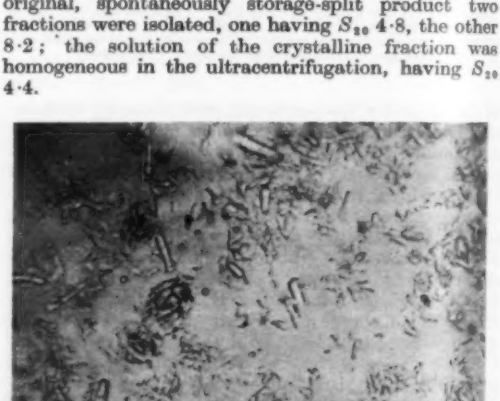

Fig. 3. Crystals in the γ-globulins prepared according to Lever

Variations of γ-globulin observed during storage are apparently caused not only by plasmin notwithstanding that this ought to be adjudged the decisive role in this reaction in plasmatic γ-globulins. A quicker splitting in products prepared from hæmolysed materials indicates the share of 'neutral' enzymes present in erythrocytes[4]. The appearance of crystalline atypical fraction seems to indicate the analogy with γ-globulin fraction III isolated by Porter[5] by papain-digestion of rabbit γ-globulin, but regardless of the crystalline structure.

Further details of our observations will be published elsewhere.

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Effect of Amethopterin and 6-Mercaptopurine on the Pattern of Oxygen Tension Measurements in the Fluid Phase of Serum Agar Cultures of Bone Marrow Flecks

We have recently described an electro-chemical method of measuring the oxygen tension in the fluid phase of serum agar cultures of bone marrow flecks[1]. Oxygen tension decreased, at first fairly quickly and then more slowly over a period of three days. This was ascribed to the utilization of oxygen by the surviving cells. In this communication we describe some preliminary experiments upon the effect of amethopterin and 6-mercaptopurine on the oxygen tension pattern of similar cultures. At the beginning of the experiments the culture fluid was equilibrated with air containing 5 per cent carbon dioxide; the carbon dioxide was required to keep the *p*H of the fluid in the physiological range. After a number of hours the fluid was replaced either with fresh medium alone or with medium containing the drugs. As this fluid was also equilibrated with the air-carbon dioxide mixture, the oxygen tension rose but not usually to the starting level. This may have been due to the mixing of the fresh medium with the relatively anoxic serum agar also present in the culture chamber. Thereafter, in the normal controls the oxygen tension continued to fall until the experiment was concluded. This is illustrated by the control curve seen in Fig. 1.

When a medium containing a 1 m*M* concentration of amethopterin was added to the culture of normal marrow there was a definite alteration in the pattern which is illustrated in Fig. 1. After a latent period of about 8 hr. the curve flattened; this we think is due to interference with the normal oxygen metabolism of the cells. It did not occur in 0·25 m*M* concentrations; 0·5 m*M* concentrations showed an intermediate effect.

The findings with 6-mercaptopurine were comparable. We found that a 6-m*M* concentration caused a flattening in the oxygen tension curve after a latent period of about 20 hr.; this did not occur with 2-m*M* concentrations and 4-m*M* concentrations had an intermediate effect.

The oxygen tension patterns of the fluid phase of serum agar cultures of marrow flecks from a case of acute leukæmia have also been studied. The patient, a woman aged forty, was admitted to hospital with pneumonia. The peripheral blood showed a subleukæmic picture, and the bone marrow was stuffed with primitive leukæmic cells. After a stormy course and treatment with antibiotics, steroids and 6-mercaptopurine a remission was induced. This lasted 5 months, after which relapse and death occurred. At the time of the cell culture the marrow showed predominantly primitive leukæmic cells. Four cultures were made without the addition of drugs, and the oxygen tension patterns resembled those found in normal marrow. 6-mercaptopurine was added to three cultures in concentrations of 6, 4 and 2 m*M*, respectively. The curve obtained with the culture containing the 6-m*M* concentration showed a levelling after about 10 hr.; those obtained from the 4- and 2-m*M* concentrations continued downwards. Amethopterin was added to three cultures in concentrations of 1, 0·5 and 0·25 m*M* respectively; in none of these was a levelling effect seen.

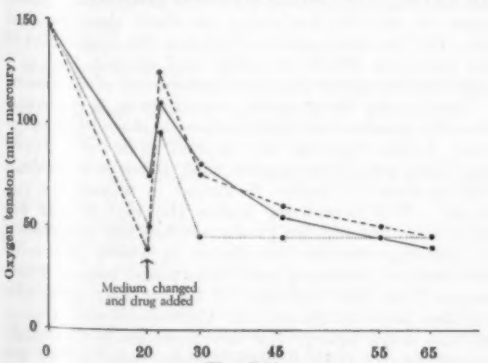

Fig. 1 ●—●, No drug control; ●···●, 1 m*M* amethopterin; ●– – –●, 0·25 m*M* amethopterin

These preliminary results are of interest because they could form the basis of an *in vitro* test of the sensitivity of leukæmic marrow cells to various chemotherapeutic agents. This would be based on the hypothesis that the concentrations of drugs which would inhibit the oxygen uptake of sensitive cells might not affect cells which were resistant.

We are grateful to Messrs. Lederle and Burroughs Wellcome for a supply of drugs.

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PLANT PHYSIOLOGY

Effect of Gibberellic Acid on Flower Differentiation in Petkus Winter Rye

It is known that application of gibberellic acid causes flowering of many biennials (*Hyoscyamus*, *Apium*, *Brassica*, *Daucus*, etc.)[1]. In these cases gibberellic acid substitutes for cold (vernalization) treatment. Treatment of winter cereals (Petkus rye[2], barley[3], winter wheat[4]) with gibberellic acid, on the contrary, has been found to have no effect on the induction of normal flowers. Chaylakhian[5] has reported that treatment of non-vernalized oats, variety Victory, with gibberellic acid, under short-day conditions, caused formation of an increased number of developed panicles. This was not the case under long-day conditions. Paleg and Aspinall[3] have reported that treatment of barley with gibberellic acid for several weeks immediately following emergence results in the formation of abnormal spikes.

The experiments reported here are concerned with the normal flower formation of Petkus winter rye by application of gibberellic acid. This cereal is, under the growing conditions of our experiments, a cold-requiring plant.

It has been found that, to be successful, treatment must occur at a particular stage in the development of the plant.

All the experiments were performed under the controlled greenhouse conditions of the Earhart Plant Research Laboratory. A description of this laboratory and the experimental facilities available in it has been given by Went[6].

Plants of winter Petkus rye, grown under a constant temperature of 17° C., both in short-day (8 hr.) and long-day (16 hr.) regimes, were sprayed with

water solutions of gibberellic acid (200 p.p.m.). The plants of each day-length regimen were divided into three groups: (1) controls, sprayed with deionized water; (2) spray treatment started when the plants had five visible leaves; (3) spray treatment started when the plants had ten visible leaves. Each group was composed of 18 plants. The plants were sprayed for seven successive days after which they were permitted to develop without further treatment.

75 days after sowing the seed the plants were dissected and the stage of development of the growing point of each main shoot recorded. For the five-leaf stage this dissection occurred 48 days after the end of treatment; for the ten-leaf stage, 12 days after treatment. In order to evaluate the stage of development, an arbitrary numerical scale from 0 to 4 was used, in which stage 0 is the early vegetative apex and 4 is a complete flower just prior to full development of the awns.

The results (Table 1) show that winter Petkus rye which has not been vernalized can be induced to form normal flowers by the application of gibberellic acid. The application of the hormone apparently must, however, occur at a proper time in the development of the plant (ten-leaf stage). Applied before this time, gibberellic acid does not induce flowering. It is, on the contrary, inhibitory. The typical effect of gibberellic acid on growth in grasses is found in both the early and the later applications of the acid, that is, elongation of the internodes and narrowing of the leaves.

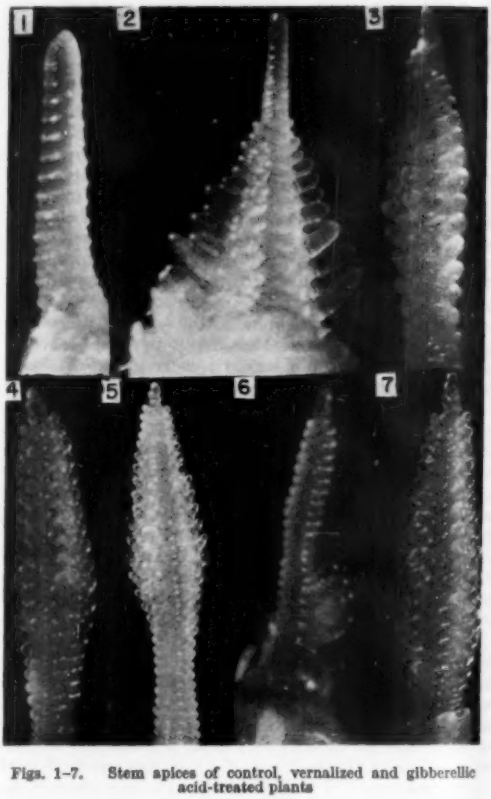
Table 1. Effect of Gibberellic Acid on the Flowering Behaviour of Winter Petkus Rye

Treatment with 200 p.p.m. at the	Long day	Short day
Five-leaf stage	Veg./abnormal* 18 plants	Veg./abnormal 13 plants* Flower/abnormal 4 plants 17 plants
Ten-leaf stage	Flower primordia (stages 3–4)* 18 plants	Flower primordia (stage 4 to head)* 18 plants
Control (water spray)	Vegetative* 17 plants	Flower primordia (stages 3–4) 10 plants Veg. primordia 8 plants 18 plants

* See Figs. 1–7 for a description of these developmental stages.

Figs. 1, 2 and 3 are photographs of typical apices of the three treatments on long day. Fig. 1 shows the apex of a control plant after 75 days of growth. The plants are all vegetative. Fig. 2 shows the effect on the growing point of applications of gibberellic acid at the five-leaf stage. It can be seen that the lower part of the apex is abnormal with great numbers of developing tillers. The upper part is normally vegetative. We consider this portion of the apex as having been developed after the effect of the applied gibberellic acid has ceased. Fig. 3 shows a typical apex from a plant treated after the tenth leaf has unfolded. This is a normal flowering apex as can be seen by comparison with Fig. 4, which represents an apex dissected from a vernalized control plant at the same stage of development as that shown in Fig. 3.

Figs. 5, 6 and 7 represent apices dissected from plants grown on short days. Fig. 5 pictures the unvernalized apex of a control plant; because of the promotive effect of short days on flower formation in unvernalized winter rye (short-day vernalization: see ref. 7), this apex is further advanced than comparable apices of plants grown on a long-day regimen and shows an early stage of flower development. Fig. 6



Figs. 1–7. Stem apices of control, vernalized and gibberellic acid-treated plants

shows the effect of gibberellic acid treatment at the fifth-leaf stage. These plants show a distinct abnormality at the base of the apex with an early stage of a normal flower at the top of the apex. Fig. 7 shows the effect of gibberellic acid treatment at a later stage (10 leaves).

On short day there is a significant difference between controls and those plants treated at the ten-leaf stage in the number of plants which flowered at the time of dissection, as well as the stage of development. Only 5 per cent of the controls had well-developed flower primordia, while all the plants treated at the ten-leaf stage had advanced flower primordia. Treatment at the five-leaf stage on short days resulted not only in abnormalities, but also the upper part of the apex, on which flowering had started, was at a significantly earlier stage in development of the flower.

Considering the long-day experiments, it is obvious that the plants must reach a specific stage of development before they can be induced to flower. Purvis and Gregory[7,8] have shown that there is a minimal leaf number (7) before flowering in winter rye can occur. The primordia above the eighth leaf are indeterminate and can produce either leaf or spikelet. In our experiments the plants in which gibberellic acid caused flowering had 10 exposed leaves, which means that the number of leaves and primordia totalled between 20 and 25. Gibberellic acid applied at this stage affects the development of the labile primordia so that flowers rather than leaves are formed. Since gibberellic acid affects flower formation in plants only after the ten-leaf stage, the acid or a

similar substance must be limiting, while 'florigen' is not.

In our experiments and especially in those of Paleg and Aspinall[2], it has been shown that applications of gibberellic acid at an early stage in the development (before the ten-leaf stage) will result in inhibition of flowering. Therefore this early application must either inhibit the formation of, or destroy, the florigen.

Hence, at least two things must happen during vernalization: (1) 'florigen' must be formed, or its formation induced after removal from cold; (2) a gibberellic acid-like substance must also be made, accumulated, or its formation induced so that it is formed soon after the plant is removed from the cold.

A more detailed report of this work is being prepared for publication elsewhere.

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Effect of Gibberellin on the Flower Initiation and Stem Extension in Petkus Winter Rye

The cold requirement for flower formation in biennial *Hyoscyamus niger* was shown by Lang[1] to be replaceable by application of gibberellin, but no flowering response was observed at that time in either Petkus winter or spring rye similarly treated[2]. In 1958, I attempted to supplement partial vernalization in Petkus winter rye by a single injection of gibberellic acid (10 p.p.m.) through the third leaf. No effect on flower initiation was observed. Stem extension was unaffected in the unvernalized controls but considerably enhanced in the partially vernalized series. In 1959, further work was carried out to determine whether the lack of response was due to the low concentration of the single application of gibberellic acid, or whether stimulation of stem extension demanded some prior degree of flower initiation.

Unvernalized winter rye was injected with gibberellic acid at 20 p.p.m.; this was done by feeding through the severed tips of mature leaves. Three groups received single injections to the third, sixth and ninth leaves, respectively, at the time when these had reached full expansion (that is, approximately at intervals of 3 weeks). In a fourth group the sixth and ninth leaves were injected as they reached maturity, while in a fifth group the third, sixth and ninth leaves were treated in turn.

The results are presented in Table 1, in which the effects on progress of flower initiation as 'scores' attained 93 days after planting, and on stem extension, are shown. 'Scores' less than 21 indicate that flower initiation (double ridges) had not been attained, while higher values mark progress beyond this stage.

Stem extension. A single injection at the third-leaf stage had no effect, as was also found in 1958. Single injections at the sixth or ninth leaf-stages resulted in limited stem extension (3 cm. in each case) apparently attained at a higher rate when the ninth leaf was treated. Multiple injections produced an effect rather more than proportional to the number of leaves injected. Thus an injection of the third leaf, apparently ineffective when this leaf alone was treated, intensified the response to treatment of later leaves.

Flowering response. The flowering response was entirely different. Injection of the third leaf resulted in a very small increase in 'score' just below the significant level. Injection of the sixth leaf resulted in a significant increase, which was further augmented by injecting the ninth leaf. Multiple injections effected no greater increase than that following a single injection of the ninth leaf; indeed, the triple injection decreased the score significantly below that due to the single injection.

Although the plants were not grown to maturity, it was evident that those injected through the ninth leaf alone would have produced ears free from morphological abnormalities. Where earlier leaves had been injected in addition, very many abnormalities were found, such as the formation of subsidiary ears from basal primordia ('miracle' ears), leafy outgrowths below the spikelets, or shortening and distortion of the peduncle[3]. This was also true to a lesser degree where the sixth leaf had been injected, but was not, of course, apparent in the undifferentiated apices of plants treated at the third leaf. These effects correspond closely with those obtained by Caso *et al.* (preceding communication).

Thus it appears that gibberellin has no direct effect on flower induction in Petkus winter rye, and therefore cannot replace low temperature in this respect; the accelerating effect on flowering would appear to operate at some post-inductive stage. The stimulus to stem extension only occurs either just before or after flower initiation has taken place.

Table 1. Effect of Injecting Unvernalized Winter Rye with Gibberellic Acid (20 p.p.m.) through Different Leaves

No. of injections Leaf injected	0 None	1 3	1 6	1 9	2 6 + 9	3 3 + 6 + 9
Score at time of injection		7·1 ± 0·34	9·6 ± 1·19	13·0 ± 0·91		
Score at 93 days	17·8 ± 0·39 *←NS→*	19·2 ± 0·78 *←SS→*	23·4 ± 0·66 *←SS→*	27·8 ± 0·49 *←NS→*	27·8 ± 0·40 *←S→*	26·3 ± 0·58
Stem length at 93 days (cm.)	0	<0·5	3·0 ± 0·39	3·1 ± 0·50	7·7 ± 1·22	13·1 ± 1·80

N.S., Difference not statistically significant; *S.*, significant, $P = 0{\cdot}05$; *S.S.*, highly significant, $P = 0{\cdot}01$.

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BIOLOGY

Rare-Earth Fatty Liver

The biological behaviour of the rare earths has recently become a question of increasing importance[1]. Early recognition of the prominent occurrence of several of these elements in fission products[2] stimulated a wide scope of distribution studies in animals and, to a limited extent, in man. Yet, we could find among these studies no mention of a specific biochemical or histological effect except for a brief communication from this laboratory on fatty liver degeneration[3] and some earlier reports on rare earths as anticoagulants[4].

We recently characterized the occurrence of an acute fatty infiltration of liver caused by the stable rare earths of lower atomic number (lanthanum to samarium) when they are administered intravenously to rats. The fatty response, which is represented by an increase in the neutral esterified fatty acids with little change in the phospholipid or cholesterol content, is influenced by a variety of hormonal factors[5]. This communication gives results that show the effect of time on recovery of the rat liver from the lipid disorder and the liver lipid response of certain other species when a rare earth is administered.

Female rats (Carworth Farm-Nelson) weighing 180–240 gm. were used in the time study which is summarized in Fig. 1. Pertinent information for the various species used in the other study is included in Table 1 along with the results. All animals in both experiments were fasted 24 hr. before they were killed. The materials and the methods of extraction and measurement of total liver lipids were the same as those used previously[5].

Fig. 1 shows that the maximal increase in liver lipids after a 2 mgm./kgm. dose of stable cerium occurs at 2–3 days. Six days after receiving the cerium injection the liver lipids returned to normal. The recovery period, the fourth to fifth day, shows a wide biological variation. Some preliminary studies indicate that the amount of cerium in the liver remains

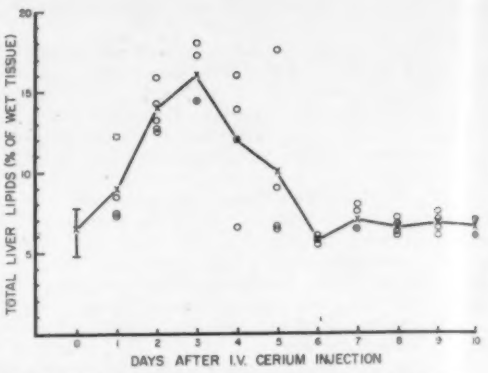

Fig. 1. Total liver lipids as a function of time after intravenous cerium (2 mgm./kgm. in rats). ○, Individual rat value; ⊙, two individual rat values are identical; ×, mean value. The range is shown for the control group (0 days) with the mean value based on analyses of 18 rats

at essentially the same high level (about 70 per cent of the administered dose) at least from 4 hr. to 12 days (Rafter, J. J., Kyker, G. C., and Snyder, F., unpublished results); that is, there is no tendency for the liver cerium concentration to decrease during the recovery phase.

Because cerium appears to be rather representative for studying the behaviour of the first part of the rare-earth series, this element has been used to survey the lipid response in both males and females of other species under the same conditions that had caused the fat disturbance in rats. The results in Table 1 show a significant elevation in liver lipids for rats (at 3·5 mgm./kgm. for females and 14·0 mgm./kgm. for males), mice (at 7·0 and 14·0 mgm./kgm. for females), and hamsters (at 7·0 and 14·0 mgm./kgm. for males). No fatty livers were seen in any of the other species except in two male rabbits that had 14·7 and 18·2 per cent liver lipids at 48 hr. after a 3·5 mgm. cerium dose. This variation in

Table 1. Liver Lipids in Different Species 48 hr. after a Single Intravenous Cerium Injection

Species	Sex	Cerium (mgm./kgm.)				
		0	3·5	7	14	28
		(per cent lipids in wet tissue*)				
Rat (200–267 gm.)	M	5·34 (3)	7·93 (3)	9·54 (3)	9·16† (3)	
		5·03–5·69	6·47–10·74	8·35–10·29	8·33–10·33	
	F	6·11 (7)	11·35† (12)			
		4·38–7·40	9·39–14·53			
Mouse (13–20 gm.)	M	7·09 (2)	6·99 (3)			
		7·02–7·16	5·57–9·62			
	F	6·01 (6)	9·01 (7)	17·52† (4)	17·00† (4)	
		4·67–7·42	5·51–12·67	9·47–24·53	15·49–20·73	
Hamster (80–127 gm.)	M	6·39 (7)	6·91 (4)	12·35† (5)	8·90† (4)	6·22 (7)
		5·34–7·25	4·70–8·04	7·26–16·15	7·06–14·00	5·35–7·04
	F	6·22 (3)	5·09 (4)	5·59 (3)	7·27 (4)	5·14 (3)
		6·08–6·43	4·76–5·58	5·34–6·03	4·97–9·90	4·55–5·60
Rabbit (1,910–2,420 gm.)	M	6·38 (5)	9·39 (6)	7·51 (3)	6·50 (3)	
		5·02–9·36	5·41–18·24	5·00–9·34	5·69–7·51	
	F	5·44 (6)	7·34 (7)	5·25 (3)	4·87 (3)	
		4·39–6·98	5·35–11·71	4·48–6·50	4·68–5·08	
Guinea pig (315–708 gm.)	M	8·09 (9)	7·05 (4)	7·08 (5)	7·13 (4)	7·82 (5)
		5·42–10·89	6·10–7·87	5·40–10·35	6·33–7·50	7·08–9·57
	F	7·40 (9)	6·49 (4)	8·77 (5)	8·23 (5)	7·71 (6)
		4·71–9·76	4·80–8·25	5·47–12·69	6·89–9·56	6·79–9·12
Chicken (1,230–1,770 gm.)	F	5·06 (8)	5·16 (6)	4·94 (5)	5·03 (4)	
		4·69–5·40	4·57–7·10	4·60–5·31	4·86–5·28	
Dog (8,000–12,000 gm.)	F	5·46 (1)	4·52 (1)		7·70 (1)	4·00 (1)

* The tabulated values represent consecutively the following: (1) the average of analytical results on individual animals in a group; (2) the number of animals in a group (shown in parentheses); (3) the range of observations on individual animals (hyphenated).

† In the interpretation of the difference between the mean of the control group (0 mgm./kgm.) and the cerium-treated groups (3·5–28 mgm./kgm.) of that species, the test of significance was applied to all the results. The standard deviations and the P values are not tabulated along with the results because of the small number of animals in the groups (220 animals; 48 groups). The dagger does signify for six groups (rats, mice and hamsters) a highly significant difference ($P < 0.01$) when interpreted by usual procedure in statistical analysis.

response among the seven species that were studied may be due either to biochemical species differences or to differences in time of response.

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Quantitative Estimations of Populations of the River Crab, *Potamon* (*Potamonautes*) *perlatus* (M. Edw.), in Rhodesian Trout Streams

THE river crab is the largest invertebrate to occur in Rhodesian trout streams, but standard quantitative sampling methods reveal insignificant numbers of this animal. As has been suggested by Van Someren in Kenya[1], it is doubtful if anglers have been correct in assuming that the crab is an important trout-food item. Nevertheless, preliminary Rhodesian studies[2] indicate that it occurs in trout stomachs with a median percentage frequency occurrence of 13·1; furthermore, there is a 100·0 per cent frequency and 97·5 per cent volumetric occurrence in droppings of the clawless otter, *Aonyx capensis* Schinz. These figures are based upon slightly more than 1,000 stomach contents, and exactly 1,000 otter samples. In such circumstances the river crab takes on a certain economic standing, even if ignored as a competitor or scavenger.

The known Rhodesian habitat of *P. perlatus* (var. *inflatus*, *depressus*, *sidneyi*) can be described as mountain streams having an altitude ranging from 5,000 to some 6,800 ft. a.m.s.l. (1,520–2,070 m.), although the species may well be found both above and below these limits in addition to occurring in weirs or dams built upon the streams. In these waters the crabs can be taken in funnel-entrance traps baited with fresh fish, and if such traps are enclosed within a screened but typical section of stream then the number of animals captured decreases each time the traps are cleared. Thus it is possible to apply the population estimation method of De Lury[3], in which numbers of animals captured are plotted graphically against an ordinate, and total catch to date against an abscissa. Extrapolation of a fitted straight line to an intersection with the abscissa provides an estimation of the population present at that time. In practice, traps are cleared each 24 hr., and there is little difficulty in relating the results of test areas to the total area of stream-bed under consideration.

Chemical destruction of soft and hard parts of adult crabs suggests that the wet weight of soft parts represents but about 34·0 per cent of total wet weight. In assessing populations as potential fish or otter food this factor should be taken into account. It may be recalled that Allen[4] treated the mollusc *Potamopyrgus* in similar fashion, although that worker dealt with dry weights.

Trapping is selective in that small specimens having a maximum carapace width of less than 17·0 mm. are not attracted to baits so far tested. However, the larger size groups consistently taken in traps can usefully be discarded when taken in orthodox net or dredge samples. In impoverished African trout waters the occurrence of a single large crab can have a grossly distorting effect upon the collection, and when such large-size animals appear it is an asset to be able to ignore their occurrence. The small-size group, in Rhodesian trout streams being less than 17·0 mm. carapace width, have less distorting effect and may be incorporated in orthodox sampling results. During practical field work it is therefore suggested that trapping forms a valuable adjunct to a series of dredge and net samples, provided that the degree of selectivity is determined and allowed for.

Preliminary results at Inyanga, the main trout area of Central Africa and having a central point at about 32° 44′ E., 18° 17′ S., indicate that *perlatus* specimens subject to trapping range from 17·3 to 45·0 mm. maximum carapace width. The range for males is 22·0–45·0 mm., and that for females 17·3–37·5 mm. The median wet weight of all specimens taken, after drying-off of all surplus moisture, ranges from 7·375 to 10·510 gm. There is some evidence that smaller size groups pertain where other trout food organisms are in poor supply; but in these areas the crabs are infested with a small greenish leech, normally some 10·0 mm. in length when extended, and customarily found at the bases of the legs. To date no phoresic associations with the crabs have been detected, such as the phoresis between *Potamon niloticus* and immature stages of *Simulium neavei*, and between a crab species and *S. nyasalandicum*[5]. Calculated populations of river crabs in Rhodesian trout streams range from 3,049 to 5,227 per acre (7,530–12,910 per hectare). The wet weight per acre ranges from 49·5 to 121·2 lb. (55·5–135·8 kgm. per hectare), and the wet flesh weight from approximately 16·82 to 41·2 lb. per acre (about 18·86–46·15 kgm. per hectare).

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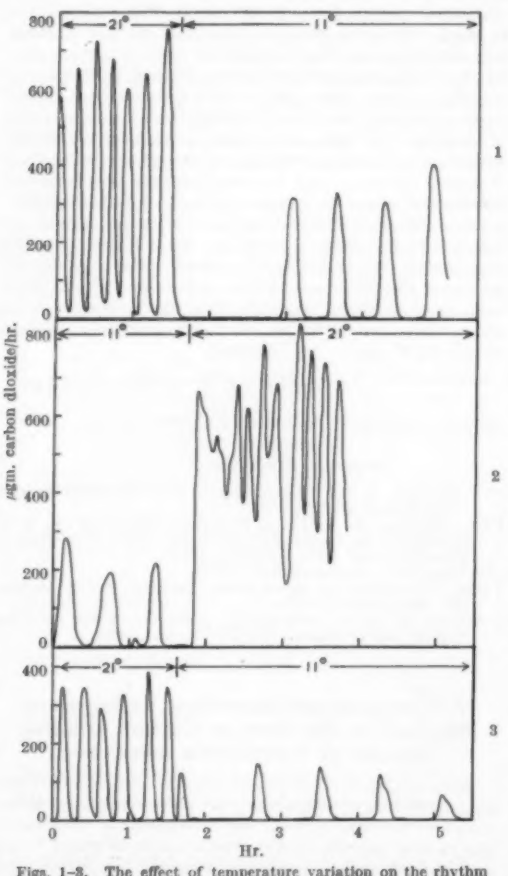
A Temperature-dependent Endogenous Rhythm in the Rate of Carbon Dioxide Output of *Periplaneta americana*

Ball, Dyke and Wilkins[1] and Wilkins[2] developed an apparatus, incorporating an infra-red gas analyser (Grubb-Parsons, *S.B.*1), for continuously estimating the rate of carbon dioxide metabolism of plant tissue maintained under constant external conditions for 5–10 days. Using this apparatus the rate of carbon dioxide output of individual adult cockroaches was

determined. The insects were kept in 12 : 12 hr. alternations of light and darkness and, at the commencement of an experiment, were transferred to a small flask which was placed in darkness and at a constant temperature at the end of a light period. The records presented here were derived from one insect, but five other cockroaches gave closely similar results.

In a stream of air initially free from carbon dioxide (relative humidity 80–90 per cent), darkness and at 21 ± 0·1° C. the period of the rhythm was 14·1 ± 0·8 min. (Fig. 1). On lowering the temperature to 11 ± 0·1° C. carbon dioxide output ceased for 76 min. and then the rhythm recommenced with a period of 35·8 ± 1·4 min. and with an amplitude approximately half that exhibited at 21° C. In an initially carbon dioxide-free air stream the period shows a $Q_{10} = 2·5$. The reverse procedure, increasing the temperature from 11° C. to 21° C., reduced the period from 35·8 ± 1·4 min. to 12·6 ± 1·9 min. (Fig. 2). A lag of 30 min. occurred between the change in temperature and the resumption of the rhythm, and the peaks during the first hour at the higher temperature were somewhat irregular. This may account for the slightly shorter period and larger standard error.



Figs. 1–3. The effect of temperature variation on the rhythm of carbon dioxide output of an adult cockroach maintained in darkness and in either a carbon dioxide-free (1 and 2) or normal (3) air stream. For explanation, see text

In a stream of normal air, darkness and at 21° C. the period of the rhythm was 16·2 ± 2·0 min. (Fig. 3). On reducing the temperature to 11° C. carbon dioxide emission ceased for 45 min. and afterwards recommenced with a period of 47·3 ± 0·03 min. In normal air the period shows a $Q_{10} = 2·9$ and again the amplitude was halved by the temperature reduction.

Removal of carbon dioxide from the air stream has no significant effect upon the period of the rhythm at 21° C., but at 11° C. the period is significantly longer in a normal air stream than in a carbon dioxide-free air stream. The rhythm was not inhibited or the period modified when insects maintained in an initially carbon dioxide-free air stream were illuminated with white light at an intensity of 3,000 lux.

These results contrast with those of Punt[3], who showed that *P. americana* lacks a rhythm of carbon dioxide emission, output being irregularly continuous in darkness, at 15° C. On the other hand, a rhythm of carbon dioxide output has been reported in the locust[4] in which the period was 2·5 min. in darkness, at 32° C. and in an initially carbon dioxide-free air stream. The locust exhibited the rhythm for only 24 hr. after shedding the last hopper skin. In contrast, the rhythm persisted for at least 4 days in the adult cockroaches used in this work. The period of the rhythm of emission of carbon dioxide in silk-worm pupæ has been shown to vary between 45 and 120 min. at 25° C.[5,6].

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'Watery Stipe' of Cultivated Mushrooms

In a recent paper, Lindberg[1] described a transmissible disease of *Helminthosporium victoriae* in which the infective agent, not identified, caused a stunting of the mycelium. This condition could be transmitted to healthy cultures through contact, either by growing the two types together or by treating normal cultures with a suspension of macerated diseased mycelium in sterile water.

A very similar condition has now been found in the cultivated mushroom, associated with a disorder known to growers as 'watery stipe', 'brown disease' or 'La France disease'. The macroscopic symptoms are variable and include the death of immature sporophores which may turn brown. Different degrees of water-logging of the tissues, particularly of the stipe, also occur. In mild cases this appears as translucent streaks on the stipes, but in extreme cases the whole sporophore may be saturated and exude drops of liquid from the surface of the pileus. Sporophore distortions, such as elongated and bent stipes, are commonly found. Cropping is much below normal and ceases prematurely, this being the most serious aspect of the disorder to the commercial grower. The variable symptoms have made positive macroscopic identification impossible. The only consistent

feature which has been discovered has been the degeneration of the mycelium, which is not always apparent from a cursory examination. If, however, fragments of the mycelium are plated out on an agar medium they will, if infected, produce weak, buff-coloured adpressed colonies without rhizomorphs. Healthy mycelium produces fluffy, white, more rapidly growing colonies with rhizomorphs radiating from the point of inoculation. The cells of the abnormal mycelium are shorter than normal and have a slightly swollen appearance. No other differences have been noted.

This mycelial degeneration has been transmitted to healthy cultures using methods similar to Lindberg's. With the double inoculation method, diseased and healthy cultures grow until their mycelia ultimately come into contact, but anastomoses between the hyphæ of the two colonies have not been seen. The coalescence of the two cultures is slow, as if there were antagonism between them, which is in marked contrast to the behaviour of two healthy colonies, where many of the rhizomorphs fuse. The growth of the healthy culture is usually weaker after coming into contact with the abnormal one; but there is no visible change in the appearance of the older mycelium. However, if this (originally healthy) mycelium is subcultured, its growth is abnormal.

Experiments with macerated mycelium have so far been confined to the use of suspensions containing fairly large fragments of mycelium which are capable of regeneration. Normal mycelium dipped in such a suspension grows at less than half the speed of mycelium dipped in suspensions of healthy mycelium or sterile water. Filtration to remove these mycelial fragments also removes the infective agent.

This investigation into the cause of the stunting of mushroom mycelium continues. Tests for the presence of bacteria have given negative results so far. The presence of a virus may be a possibility, although none has been found in fungi.

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GENETICS

Self-Incompatibility System in Two Mexican Species of *Solanum*

So far the genetics of self-incompatibility has been described in only one species of *Solanum*, namely, *S. chacoense*[1]. It is an extremely variable species which occurs in north and central Argentine, Paraguay, Uruguay and south Brazil and now includes *S. caldasii*, *S. subtilius* and *S. jujuyense*[2]. *S. chacoense* has a one-gene, multi-allelic, gametophytic system of self-incompatibility similar to that which has been found in three other genera of the Solanaceae, *Nicotiana*, *Petunia* and *Lycopersicon*. Recently I have found that *S. megistacrolobum* has the same system as its sister South American species *S. chacoense* (in the press), whereas the two Mexican species, *S. ehrenbergii* and *S. pinnatisectum*, have a two-gene system.

Progenies of 20 plants were grown from reciprocal crosses between unrelated clones of each species. Siblings within each family were crossed in all directions and each sibling was also crossed reciprocally with its parents. In *S. ehrenbergii* one family produced four intra-sterile groups of plants, but the family from the reciprocal cross produced six such groups. In *S. pinnatisectum* both the families from the reciprocal crosses gave six intra-sterile groups each. Different groups in one family were cross-compatible with some and cross-incompatible with other groups. There were reciprocal differences in the results of crosses between different groups.

The results obtained in the two Mexican species are conspicuous for being quite different from those usually obtained in the *Nicotiana* type system. They are explained on the basis of the following assumption. The genetics of incompatibility is controlled by two independently segregating loci each with a series of multiple alleles. Pollen determination is gametophytic and there may be individual action or epistasis between the alleles of the two series. In the style there is individual action of *S* alleles. A pollen grain is incompatible on a style when either one or both of its two alleles (of two different series) are also present in that style. This system may prove to be of general occurrence in the Mexican species and limited to them. It has been found to occur also in *Physalis ixocarpa*[3], a native of Mexico and Central America. In contrast, the 1-locus system appears likely to be confined to the species of South America.

The occurrence of the 1- and 2-locus gametophytic systems of incompatibility in the same genus *Solanum* is of considerable evolutionary interest. There is enough evidence to suggest that the 1-locus *Nicotiana* type of gametophytic incompatibility, occurring in a large number of flowering plants, was the first system of incompatibility to develop, and evolved early in the evolution of angiosperms[4]. The sporophytic system[4] and the 2-locus gametophytic system[5] were both derived from this system. It also seems justifiable to assume that the 2-locus system was able to evolve in the Mexican diploid tuberous *Solanums* because of their geographical isolation as a result of the separation of North and South Americas.

Some of the biological forces which might have led to this genetic distinction between the species of the two groups can be suggested. Several investigators[6], on the basis of cytological studies, have concluded that the basic chromosome number in the family Solanaceae is 6 and not 12, which occurs in most of its species. If it is assumed that the present basic number 12 for a large part of this family has been derived by doubling of the original basic number six, very early in the evolution of this family, then the two *S* loci in the Mexican species are the result of the duplication of the original *S* gene. However, the possibility of a segmental interchange bringing about duplicate *S* loci cannot be ruled out.

Whatever might have been the origin of two *S* loci, in the beginning they must have caused self-compatibility due to the competition interaction between the *S* alleles in the pollen[7]. The restoration of self-incompatibility could then have occurred by different methods in the two groups of geographically isolated Solanaceous species. (1) In the South American species having a 1-locus system of incompatibility, the restoration of incompatibility occurred by a selection for the loss of function of one of the two *S* loci—a phenomenon which seems to have occurred in some of the known tetraploid species (for example, *Trifolium repens*[8]) of other families. (2) In the Mexican species having two *S* loci, the restoration of incompatibility

occurred by a selection against competition interaction between the *S* alleles in the pollen, thus retaining two functional *S* loci. The absence of competition interaction in the diploid pollen of *Physalis ixocarpa*[3] seems to support this hypothesis. The reason why the Mexican group of *Solanum* species should have evolved differently in this respect than their sister species in South America is, however, not clear.

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PSYCHOLOGY

Intelligence, Personality and Brain Rhythms in a Socially Isolated Community

SIGNIFICANT correlations between alpha rhythm in the electroencephalogram and ratings of the temperament variable primary-secondary function have been attributed to genetically determined individual differences in a central nervous excitability characteristic[1,2]. Since both alpha frequency[3] and vocabulary score[4] of the Wechsler Adult Intelligence Scale were reported as positively correlated with speed of perception, it seemed possible that performance on the Wechsler Adult Intelligence Scale might be influenced by primary-secondary function. This argument was supported by the finding in 34 subjects of a significant correlation ($r = 0{\cdot}51$, $P < 0{\cdot}01$) between alpha frequency and total score on the Wechsler Adult Intelligence Scale. Further analysis suggested that the Scale measures non-intellective in addition to intellective aspects of intelligence, and

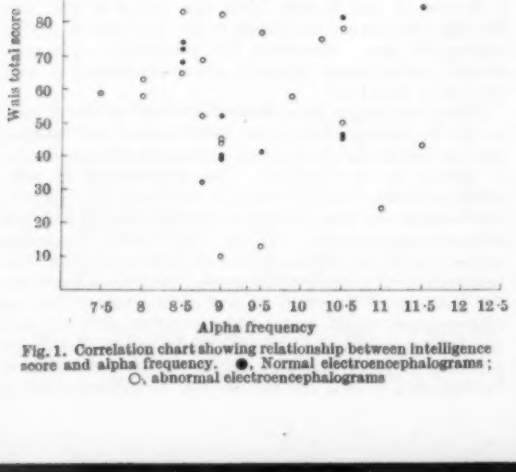

Fig. 1. Correlation chart showing relationship between intelligence score and alpha frequency. ●, Normal electroencephalograms; ○, abnormal electroencephalograms

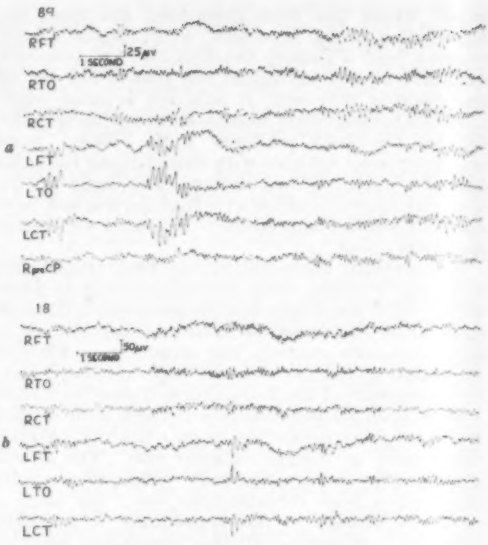

Fig. 2. Electroencephalogram abnormalities from two members of a socially isolated community, neither with any obvious neuropsychiatric symptom or known abnormal history: *a*, male, 64 yr., intelligence quotient not measured. Bilateral independent focal bursts from temporal lobes, more marked on left, frequencies mainly between 8 and 12 c./sec. with some theta rhythm; *b*, male, 56 yr., intelligence quotient 75. Focal spikes and sharp waves from left temporal lobe, also runs at 10–13 c./sec. and traces of theta rhythm. *R*, Right; *L*, left; *F*, frontal; *T*, temporal; *O*, occipital; *C*, central; *P*, parietal; *preC*, precentral

that the former possess a number of electroencephalogram correlates[5]. The fact that previous studies had failed to reveal correlations between alpha rhythm and intelligence was attributed to differences between the Wechsler Adult Intelligence Scale and other intelligence tests, and also to a sampling factor, it being proposed that the emergence of electroencephalogram correlates of non-intellective components of intelligence is dependent on the intellective and socio-economic homogeneity of the sample studied[6].

As a test of these hypotheses we undertook an expedition to a remote region of South Africa to study a group of white labourers from a socially isolated community of long standing, the members of which are characterized by a high degree of behavioural uniformity and generally low intelligence. Electroencephalograms were recorded from 94 men and 2 women, of whom 55 men were also given the South African version of the Wechsler Adult Intelligence Scale. A number of other psychological tests was also applied.

Two important findings have now emerged. First, as predicted, alpha frequency is significantly correlated with total score on the Wechsler Adult Intelligence Scale ($r = 0{\cdot}34$, $P = 0{\cdot}01$, Fig. 1) and with raw scores from 5 Scale sub-tests, including vocabulary. These correlations were virtually unchanged with age held constant. Secondly, 49 (51 per cent) of the 96 subjects possessed abnormal electroencephalograms, all but one because of bilateral shifting foci from the temporal lobes, usually in the form of sharp waves, spikes, or bursts of activity ranging in frequency from 2–14 c./sec. (Fig. 2). These

focal disturbances tended to be more prominent from the left side.

The correlations between alpha frequency and Wechsler scores support our hypothesis that given a homogeneous sample it is possible to identify a non-intellective component of intelligence which is related to a central nervous excitability factor. Detailed reports of distributions and interrelations between electroencephalogram variables, Wechsler scores and other psychological measures will be published elsewhere.

The finding of 50 per cent bilateral independent temporal lobe foci was quite unexpected, since this type of electroencephalogram abnormality is strong indication of organic disturbance in one or both temporal lobes, and is particularly common among certain epileptics[6]. Our sample contained one obvious case of epilepsy in the abnormal electroencephalogram group and two possibles in the normal group. There was no relation between any aspect of clinical history as elicited during interview and electroencephalogram abnormality, although no medical examination was performed. Family history revealed relatives with epilepsy in two cases in the normal electroencephalogram group and five in the abnormal group. This difference is not significant, although it seems that the incidence of epilepsy in this community is higher than in the rest of the Union of South Africa. Electroencephalogram abnormality was unrelated to age (Table 1), although the mean age of the abnormal group was significantly lower than the normal group (36 years as opposed to 43).

Table 1. Age Distribution: Normal and Abnormal Electroencephalograms

Age group	18–20	21–30	31–40	41–50	51–60	61–70	71–73
Normal electroencephalogram	0	8	8	16	8	4	2
Abnormal electroencephalogram	2	10	12	11	8	6	0

Foremost among possible explanations of the high incidence of electroencephalogram abnormality is birth injury, in view of the inadequacy of obstetric facilities in this region. In addition, malnutrition is common and may be critical, since we found that 36 per cent of a group of African infants with kwashiorkor exhibited temporal lobe foci[7]. Another possibility relates to the effects of inbreeding, known to have been prevalent in this community for many generations. In the words of the local district surgeon, "these people have remained a community within a community, merrily producing misfits of variation". Unfortunately, the need to secure the confidence of our subjects rendered it impossible to obtain any direct information concerning interfamilial marriages, but it was notable that a number of men were married to women with the same maiden name, and among the 91 male subjects showing neither filial nor fraternal relationship the total number of surnames was only 44, of which one occurred 11 times, one 8, three 5, two 4, three 3, and eight twice. This may be compared with 100 members of this Institute, among whom the total number of surnames is 90, of which three are repeated thrice and four twice. These figures support the contention that inbreeding has been prevalent among the population from which our experimental sample was drawn. That the high incidence of electroencephalogram abnormality may in some way be related to a genetic influence cannot therefore be excluded.

The mean intelligence quotient was 75, standard deviation 15, the distribution being skewed towards the lower end of the scale, with 15 mental defectives and 17 borderline mental defectives. However, although the mean intelligence quotient of the abnormal electroencephalogram group was 10 points lower than that of the normal electroencephalogram group, this difference is not significant ($t = 1{\cdot}29$, $P > 0{\cdot}20$). It is therefore unlikely that the low standard of intelligence and the high incidence of electroencephalogram abnormality are directly related consequences of a common factor.

Although distinctive personality attributes and mental symptoms have been commonly reported with electroencephalogram temporal lobe disturbances[6,8], we noted no such differentiating behavioural reactions among our normal and abnormal electroencephalogram groups. However, it should be stressed that the sample is peculiar in that it is drawn from a community which exhibits highly characteristic social traits, for example, an abject satisfaction in things as they are, an absence of individuality and self respect, a blissful self-satisfaction, and a stubborn resistance against any attempt to alter their way of life. It is possible that in such an undifferentiated and socially withdrawn community the development of mental symptoms as often observed elsewhere with temporal lobe foci would be either inhibited or moulded into the cultural framework.

We hope that later we will be able to revisit this region, both to extend the investigation in the neuropsychiatric sphere and to study younger generations, whereby we might evaluate the contribution of heredity to these findings. In the meantime, the observation that 50 per cent of a group which does not complain of fits or obvious neuropsychiatric disorder yet exhibits bilateral shifting foci from the temporal lobes merits careful consideration in relation to neuropsychology.

The expedition was undertaken with our Carnegie Mobile Psychological Laboratory, our colleagues being Miss B. Kirk-Cohen, Miss M. Oertel and Mrs. J. M. Nelson.

A. C. Mundy-Castle
G. K. Nelson
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South African Council for Scientific
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[1] Mundy-Castle, A. C., *J. Nat. Inst. Personnel Res.*, **6**, 95 (1956).
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Syllabic 'Strategies' in Written Language

Language, whether written or spoken, is one of the most sensitive indices of central nervous disorder; in a variety of neuropsychiatric states the speech and writing of the patient may be the main physical manifestation of disease. A technique of language analysis has been evolved with clinical needs in mind; but an attempt has also been made to provide a neurophysiological working hypothesis to account for the main empirical findings of the method.

The subject is asked to write 'about a page' on any topic of his own choice. The words written are then counted according to their length in syllables (S), and if p_s is the proportion of S-syllable words in the utterance, a quantity termed the 'avoidance' (A_s) is computed for each word-length. Avoidance is defined by the equation : $A_s = -\log p_s$.

The avoidances are then plotted against various simple functions of S. When it is found that A_s is an exact function of S, f(S), over the whole range of word-length employed, the sample is said to obey a pure 'strategy'. Not all, but about 25 per cent, of samples appear to show this phenomenon. Tables 1–4 contain examples of the four pure strategies so far encountered, which have been named and formulated as follows :

Linear :	$A_s = k_1.S - A_0$	Logarithmic :	$A_s = k'.\log S + A_0$
Quadratic :	$A_s = k_2.S^2 - A_0$	Log. factorial :	$A_s = k_f.\log S! + A_0$

In these equations the k are slope-constants and the A_0 are intercepts on the A_s axis, usually with the signs indicated. A_0 is found to be zero in some utterances ; in such cases the strategy requires only one constant to specify it and is therefore termed 'primitive'. In the Tables A_s are observed values while A_s* are values read off from a line drawn by eye through the points. Comparison of the two columns will indicate the closeness of fit of the strategy-equations to the data.

It seems that the brain tends to fall into a relatively limited number of patterns with respect to its spontaneous distribution of word-length. Some samples obey no obvious strategy ; others show imperfect fit to one of the pure strategies ; others can be described as obeying 'mixed' strategies which are combinations of pairs of the four pure types.

Table 1. Linear Strategy

Case *A*

S	n_s	A_s	A_s*
1	174	0·123	0·13
2	44	0·720	0·72
3	10	1·364	1·32
4	3	1·887	1·90

Case *B*

S	n_s	A_s	A_s*
1	17	0·112	0·11
2	4	0·740	0·74
3	1	1·342	1·35

Case *C*

S	n_s	A_s	A_s*
1	73	0·119	0·14
2	18	0·727	0·75
3	4	1·380	1·38
4	1	1·982	1·99

Table 2. Quadratic Strategy

Case *D*

S^2	n_s	A_s	A_s*
1	65	0·132	0·14
4	20	0·644	0·64
9	3	1·467	1·47

Case *E*

S^2	n_s	A_s	A_s*
1	21	0·140	0·14
4	7	0·617	0·63
9	1	1·462	1·46

Case *F*

S^2	n_s	A_s	A_s*
1	119	0·109	0·11
4	31	0·693	0·70
9	3	1·708	1·70

Table 3. Logarithmic Strategy

Case *G*

$\log S$	n_s	A_s	A_s*
0	96	0·093	0·10
0·30	17	0·845	0·84
0·48	6	1·297	1·29

Case *H*

$\log S$	n_s	A_s	A_s*
0	32	0·128	0·10
0·30	8	0·730	0·75
0·48	3	1·156	1·13

Case *I*

$\log S$	n_s	A_s	A_s*
0	41	0·095	0·07
0·30	7	0·863	0·80
0·48	2	1·407	1·37
0·60	1	1·708	1·70

Table 4. Log factorial Strategy

Case *J*

$\log S!$	n_s	A_s	A_s*
0	119	0·194	0·25
0·30	43	0·636	0·55
0·78	20	0·969	1·03
1·38	3	1·792	1·64
2·08	1	2·270	2·35

Case *K*

$\log S!$	n_s	A_s	A_s*
0	1491	0·177	0·15
0·30	453	0·694	0·48
0·78	232	0·985	1·02
1·38	59	1·580	1·70
2·08	5	2·651	2·50
2·86	1	3·350	3·37

Résumé of Sources

Case		
A	Treated chronic schizophrenic	Standard sample.
B	Recovering mild attack of encephalitis.	Standard sample.
C	Normal subject.	Spontaneous note.
D	Treated chronic schizophrenic, depressed.	Standard sample.
E	Involutional depression.	Standard sample.
F	Depressive psychosis.	Standard sample.
G	Untreated schizophrenia.	Standard sample.
H	Depressive paraphrenia.	Standard sample.
I	"The Common Cormorant"	Published nonsense-poem.
J	Epileptic of obsessional personality.	Standard sample.
K	Essay on 'Sense-data' by philosopher.	Whole essay.

The likelihood that a subject will reproduce the same strategy on two separate occasions is still uncertain ; but several subjects who have written two samples, separated by time-intervals ranging from minutes to days, in the absence of any apparent major change in physical or mental state during the interim, have shown stability of strategy apart from variations in the constants k and A_0. However, physicochemical agents which cause acute changes in the clinical state of the brain seem able to bring about 'switches' from one strategy function to another.

The findings can be explained by the following provisional hypothesis : The utterance of language consists in the conversion of specific patterns of neural activity corresponding to conceptual states (thoughts) into other patterns capable of eliciting appropriate motor activity from the motor effectors. This recoding process is assumed to involve several distinct information-handling operations, all of which must be completed before each unit of language can be uttered ; hence the slowest operation will be rate-limiting for the overall process of uttering language units. The key postulate of the theory is that if the same operation remains rate-limiting for the whole of an utterance, the character of that operation will determine the strategy.

The assumption is also made that the avoidance (that is, statistical information) of S-syllable words is proportional to the amount of information handled by the neurones performing the operation which is rate-limiting for words of this length.

Now strategies are defined above in such a way that the *word* is the unit of language and syllables are sub-units. To specify a word it is necessary to specify both its component syllables and their order. Suppose that in a given utterance the rate-limiting process (for all word-lengths) happens to be that of ordering the syllables. A set of S syllables can be arranged in factorial S ($S!$) different orders, of which one is correct and must be selected. The information required for a discrimination of this complexity is, ideally, proportional to $\log S!$. On the above assumptions, the avoidance should be given by : $A_s = K.\log S!$. This is the equation of a primitive log factorial strategy.

In a similar way it may be imagined that other operations (such as the formulation of ideas) are required in the generation of an utterance and could become rate-limiting, so giving rise to other strategies. The constants A_0 are not explained by the theory in its simple 'ideal' form but may be due to the imperfection of the brain as a communication mechanism. Mixed strategies and strategy-switches are explained by natural extensions of the theory. A fuller account of this work is in preparation.

The collection of material has been greatly helped by a grant from the Theodore Chapin Beebe Fund. I am indebted to Dr. Hick of the Cambridge Psychological Laboratory and to Dr. Russell Davis of the Medical Psychology Laboratory for their constructive criticism and advice.

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Oct. 7.

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 15

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. T. E. Tomlinson: "Mode of Action of Dipyridyl Quaternary Salts as Herbicides".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.1), at 6 p.m.—Dr. Albert Parker: "World and National Energy Resources". (First of three Cantor Lectures on "The Fuel and Power Industries and National Prosperity". Further lectures on February 22 and 29.)

INSTITUTE OF METAL FINISHING (at the Northampton Polytechnic, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. T. K. Aiken: "Complexones in Metal Finishing".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Wing Commander E. W. Anderson: "Inertial Navigation Systems".

Tuesday, February 16

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. K. Ridgway: "Problems of Early Steam Engine Design".*

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Soviet Control Engineering Progress" opened by Mr. R. E. Clark.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. C. H. Gray: "Non-Pancreatic Aspects of Diabetes Mellitus".* (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on February 18, 23, 25 and March 1.)

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Radioactive Fall-out". Chairman: Prof. A. Haddow, F.R.S. Speakers: Mr. J. F. Loutit, Dr. W. G. Marley and Prof. K. Mather, F.R.S.

Wednesday, February 17

PHYSICAL SOCIETY, COLOUR GROUP (at the Institute of Ophthalmology, Judd Street, London, W.C.1), at 3.30 p.m.—Dr. J. D. Moreland: "Colour Discrimination, Field Size, Field Position and Acceptor Populations". 4.15 p.m.—Miss J. Cox: "Colour Vision Defects Acquired in Diseases of the Eye".

BRITISH GLACIOLOGICAL SOCIETY (joint meeting with the ROYAL METEOROLOGICAL SOCIETY, at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. Herfried Hoinkes (Austria): "Studies in Glacial-Meteorology in the Antarctic, 1957".

EUGENICS SOCIETY (at Manson House, 26 Portland Place, London, W.1), at 5 p.m.—Mr. Eliot Slater: "Galton's Heritage" (Galton Lecture for 1960).*

INSTITUTE OF PHYSICS, LONDON AND HOME COUNTIES BRANCH (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Prof. S. Tolansky, F.R.S.: "The Microstructures of Surfaces".

INSTITUTION OF ELECTRICAL ENGINEERS (at Central Hall, Westminster, London, S.W.1), at 6 p.m.—Prof. M. G. Say: "Electrical Machines" (Faraday Lecture).

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. Roger Parsons: "Adsorption on Electrodes and its Relation to the Rate of Electrode Processes".

ROYAL INSTITUTE OF CHEMISTRY (at King's College, Strand, London, W.C.2), at 6.30 p.m.—Dr. W. G. S. Parker: "Rocket Propellants".

Thursday, February 18

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. P. Dean: "Vibrational Spectra of Diatomic Chains"; Mr. D. W. Pashley: "A Study of the Deformation and Fracture of Single Crystal Gold Films of High Strength Inside an Electron Microscope".

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. G. Darnley: "Petrology of Some Rhodesian Copperbelt Orebodies and Associated Rocks".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. P. J. Hilton: "Group-like Structures".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. E. A. Vigers and Mr. R. O. Fletcher: "Electricity in the Manufacture of Hydrogen Peroxide".

UNIVERSITY COLLEGE (in the Embryology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. E. Evans-Pritchard: "Religion and the Anthropologist".* (First of two Special University Lectures in Anthropology. Further lecture on February 25.)

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. Best: "Missile Control Problems".

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. R. G. Hitchcock: "The Development of the Use of a Computer for Production Control".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. T. F. Davey: "Some Recent Chemotherapeutic Work in Leprosy" with a Discussion on some of the Problems Involved in Clinical Trials.

UNIVERSITY OF LONDON (at Wye College, Wye, Ashford, Kent), at 8.15 p.m.—Prof. M. M. Swan: "New Light on Growth".*

Friday, February 19

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. J. A. Fraser Roberts: "Medical Genetics".* (Further lectures on February 26, March 2, 4, 11 and 18.)

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Symposium on "Space Navigation".

INSTITUTE OF PHYSICS, NON-DESTRUCTIVE TESTING GROUP (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. P. Schagen: "Electroluminescent Image Intensifiers".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. E. B. Knott: "Organic Chemistry of Colour Photography".

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at the Postgraduate Medical School, Ducane Road, London, W.12, at 6 p.m.—Annual General Meeting. 7 p.m. (joint meeting with the BIOLOGICAL METHODS GROUP)—Scientific Papers.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. J. Z. Young, F.R.S.: "Eyes, Colours and Shapes".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN HEALTH EDUCATION, HUMAN ANATOMY AND PHYSIOLOGY—The Principal, Borough Road College, Isleworth, Middlesex (February 22).

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF ANIMAL PATHOLOGY, School of Veterinary Medicine, to undertake teaching and research in veterinary parasitology—Dr. J. W. Millen, Department of Anatomy, The University, Downing Street, Cambridge (February 26).

ASSISTANT EXPERIMENTAL OFFICER (with an ordinary degree or diploma in agriculture or botany)—The Secretary, Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian (February 27).

ASSISTANT LECTURER OR LECTURER IN ANIMAL HUSBANDRY in the Faculty of Veterinary Science—The Registrar, The University, Liverpool, quoting Ref. CV/T (February 29).

LECTURER OR ASSISTANT LECTURER IN THE DEPARTMENT OF CHEMICAL ENGINEERING, The Houldsworth School of Applied Science, to specialize in separation processes, fluid dynamics or thermodynamics and chemical kinetics—The Registrar, The University, Leeds 2 (February 29).

PHYSICIST OR BIOPHYSICIST, to assume responsibility for an Electron Microscope—The Secretary, The Institute of Ophthalmology (University of London), Judd Street, London, W.C.1 (February 29).

RESEARCH ASSISTANT IN THE DEPARTMENT OF PHYSICS, for work concerned with the fundamental study of electro-luminescence and related processes in single crystal phosphors—The Registrar, The University, Hull (February 29).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, BIOCHEMISTRY, PHYSICS, ENGINEERING, CHEMOTHERAPY and related subjects—The Secretary, Academic Council, The Queen's University, Belfast (March 1).

ASSISTANT LECTURER (TERMINABLE) IN BOTANY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (March 5).

ASSISTANTS (2) ONE IN MATHEMATICS and ONE IN STATISTICS; and a RESEARCH FELLOW IN MATHEMATICS—The Secretary of University Court, The University, Glasgow (March 5).

LECTURER IN RADIOCHEMISTRY—The Registrar, The University, Leeds 2 (March 5).

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Secretary and Registrar, The University, Southampton (March 5).

HENRY SKYNNER SENIOR STUDENT IN ASTRONOMY—The Secretary, Balliol College, Oxford (March 12).

LECTURER or ASSISTANT LECTURER (with an interest in applied plant physiology) and a RESEARCH DEMONSTRATOR IN THE DEPARTMENT OF AGRICULTURAL BOTANY—The Registrar (Room 22, O.R.B.), The University, Reading (March 14).

LECTURER or ASSISTANT LECTURER IN CROP HUSBANDRY at Makerere College (University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 15).

MILNE-MUNRO RESEARCH FELLOW (graduate wishing to carry out research in agriculture or cognate subject) at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 15).

CHAIR OF APPLIED MATHEMATICS in the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 31).

UNIVERSITY DEMONSTRATOR IN METALLURGY—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge (March 31).

SENIOR LECTURER (with appropriate qualifications and experience) IN BOTANY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, April 5).

ASSOCIATED ELECTRICAL INDUSTRIES FELLOW FOR RESEARCH IN THE DEPARTMENT OF PHYSICS—The Secretary-General of the Faculties, The Old Schools, The University, Cambridge (April 30).

CHAIR OF BIOCHEMISTRY in the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, April 30).

LECTURER IN PHYSIOLOGY (HISTOLOGY)—The Secretary of University Court, The University, Glasgow (April 30).

BIOCHEMIST (male, with experience of work in the steroid field) at the Sheffield Centre for the Investigation and Treatment of Rheumatic Diseases—The Group Secretary, Sheffield No. 1 Hospital Management Committee, "Lyndhurst", Nether Edge Hospital, Sheffield 11.

COCONUT AGRONOMIST (with a university degree in agriculture or botany with experience in the design and analysis of factorial experiments on crops, and preferably experience of experimental work on tropical tree crops) in the British Solomon Islands Protectorate, to supervise existing experiments and to establish a Coconut Research Centre—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.63/45/04/T.

LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY—The Secretary, University College, Gower Street, London, W.C.1.

MASTER TO TEACH PHYSICS AND CHEMISTRY in the Sixth Form, with some Scholarship work in one of these subjects—The Headmaster, Blundell's School, Tiverton, Devon.

SENIOR TECHNICIAN (male or female)—The Director, Blood Transfusion Service, Roby Street, Manchester 1.

STAINES MEDICAL RESEARCH FELLOW (not exceeding 35 years of age, of English birth, and preferably with a registrable medical qualification), for research in Oxford in one of the medical sciences, such as anatomy, biochemistry, clinical medicine, pathology, pharmacology or physiology—The Rector, Exeter College, Oxford.

THEORETICAL PHYSICIST, and an EXPERIMENTAL PHYSICIST, for duties which will involve instruction in undergraduate and graduate courses—The Chairman, Department of Physics, Assumption University of Windsor, Windsor, Ontario, Canada.

REPORTS and other PUBLICATIONS

(not included in the monthly "Books Supplement")

Great Britain and Ireland

Science Books for a School Library. Pp. 54. (London: John Murray, 1959. Published for the Science Masters' Association.) 3*s.* 6*d.* net. [812

Annual Report for 1958 (No. 71) of the Marine Biological Station of the University of Liverpool at Port Erin, Isle of Man. Pp. 58. (Liverpool: Liverpool University Press, 1959.) 5*s.* [812

Scottish Marine Biological Association. Annual Report, 1958–59. Pp. 32. (Millport, Isle of Cumbrae: Scottish Marine Biological Association, 1959.) 4*s.* [812

Annual Report of the Overseas Geological Surveys, 1957–58. By Dr. F. Dixey. Pp. iv+48+7 plates. (London: H.M. Stationery Office, 1959.) 2*s.* 6*d.* net. [812

The Nuffield Foundation. Report for the year ended 31 March, 1959. Pp. xii+201. (London: The Nuffield Foundation, 1959.) [812

Institute of Personnel Management. Occasional Papers No. 14: Operational Research and Personnel Management. Part 1 by Stafford Beer. Part 2 by R. W. Revans. Pp. ii+25. (London: Institute of Personnel Management, 1959.) 3*s.* 6*d.* [812

Henry Wiggin and Co., Ltd. Brightray-Coated Exhaust Valves. Pp. 8. (Birmingham: Henry Wiggin and Co., Ltd., 1959.) [812

An Audit of Academic Performance: a Study of the Prediction of Academic Success in the Queen's University of Belfast. By Michael Forster. Pp. 75. (Belfast: Queen's University, 1959.) 7*s.* 6*d.* [812

British Museum (Natural History). Fossil Mammals of Africa No. 17: Fossil Metacarpals from Swartkrans. By J. R. Napier. Pp. ii+18+2 plates. (London: British Museum (Natural History), 1959). 8*s.* [812

Annual Report of the Advisory Council on Scientific Policy, 1958–59. Pp. iv+14. (Cmnd. 893.) (London: H.M. Stationery Office, 1959.) 1*s.* net. [1112

Ministry of Education. 15 to 18: a Report of the Central Advisory Council for Education (England). Vol. 1: (Report). Pp. xxxi+519. (London: H.M. Stationery Office, 1959.) 12*s.* 6*d.* net. [1112

Commonwealth Relations Office. Report of the Commonwealth Education Conference. Pp. 65. (Cmnd. 841.) (London: H.M. Stationery Office, 1959.) 4*s.* net. [1112

Advisory Council on Scientific Policy: Committee on Scientific Manpower. Scientific and Engineering Manpower in Great Britain, 1959. Pp. vi+48. (Cmnd. 902.) (London: H.M. Stationery Office, 1959.) 3*s.* net. [1112

The Nature Conservancy. Report of the Nature Conservancy for the year ended 30th September 1959. Pp. vi+120+14 plates. (London: H.M. Stationery Office, 1959.) 6*s.* 6*d.* net. [1112

European Brewery Convention. Report of the Barley Committee—Trials 1958. Pp. 136. (London: Institute of Brewing, 1959.) [2212

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